行政院國家科學委員會專題研究計畫 成果報告

探討血管與缺血性腦中風疾病:不同新穎複合生醫奈米材料對血管新生之分子機轉 (SDF-1/CXCR4)及治療策略發展研究

研究成果報告(精簡版)

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執 行 期 間 ：98 年 11 月 01 日至 100 年 07 月 31 日
執 行 單 位 ：中國醫藥大學中國醫學研究所

計 畫 主 持 人：洪慧珊
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中 華 民 國 100 年 10 月 25 日

附件一

行政院國家科學委員會補助專題研究計畫 ☑成果報告 □期中進度報告

（探討血管與缺血性腦中風疾病:新穎複合生醫奈米金材料對血管新生之分子機轉(SDF-1/CXCR4)及治療策略發展研究）

計畫類別：☑個別型計畫　□整合型計畫
計畫編號：NSC －98 －2314 －B － 039 － 029
執行期間： 98 年 11 月 1 日至 100 年 7 月 31 日
執行機構及系所：中國醫藥大學基礎醫學研究所

計畫主持人：洪慧珊
共同主持人：黃志揚
計畫參與人員：林建勳

成果報告類型(依經費核定清單規定繳交)：☑精簡報告 □完整報告

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附件二

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

☑達成目標

☐ 未達成目標（請說明，以 100 字為限）

☐ 實驗失敗

☐ 因故實驗中斷

☐ 其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：☐已發表 ☐未發表之文稿 ☑撰寫中 ☐無

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3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）（以500 字為限）

先前研究發現具有奈米特徵之聚胺酯 (polyurethane; PU) 基材上之可以有效促進內細胞之移動 (migration) 速率及增殖 (prioliferation) 效應，對於受損血管組織之治療方向與策略也更進一步提供具有實用性與應用性之明確方向。此計畫中我們將內皮前趨細胞 (endothelial progenitor cells, EPCs)，培養於奈米基材上探討其影響分化能力並促進成為成熟內皮細胞之相關訊息調控機轉，期能解決內細胞的之來源不足的問題，提供體內實際內皮化之速度之體內動物實驗研究模式。

一、 成效評估

綜合上述之研究成果發現，本計畫在學術發表上，PU-Au nanocomposites 已經發表了共 1 篇 (JBMR A, 2011, In press)，目前已有一篇論文已經撰寫趨於完成 (Title: Polyurethane-gold nanocompsoites promote the maturation of endothelial progenitor cells partially through stromal-derived factor-1 α)/CXC4 signaling pathway in the vascular disease model)，正在準備投稿。

二、檢討與展望

由於在製成生醫奈米材料應用於組織過程中，血液幹細胞的來源取得及其分化增殖效應仍面臨所多限制，在外來都需要更進一步修正與開發其他合適可以替代的幹細胞來源，並且尋找除了 polyurethane 外是否有其他更好生物相容性的高分子可以做為仍工血管表面改質好的生醫材料。

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＜Introduction＞

Endothelialization is an important process of vascular and a considering a protective mechanism where the endothelial progenitor cells (EPCs) a capacity of favorable responses, including antithrombotic ability, noninflammatory responses and inhibition of intimal hyperplasia [Atherosclerosis. 2010 Sep;212(1):63-9. Epub 2010 Apr 21]. Endothelial progenitor cells (EPCs) are considered as a promising alternative to primary endothelial cells for the development of vascular grafts [J Biomed Mater Res A. 2010 Nov;95(2):600-10]. These cells meet important requirements for tissue engineering applications, namely, they have therapeutic potential, a highly proliferative and antithrombogenic behavior, and can be obtained easily from peripheral blood and human cord blood. In 1997, Asahara *et al.* characterized EPCs in human peripheral blood using magnetic bead selection [Nat Med 2001; 7: 412-3]. EPCs have been implanted into various ischemic tissue models, for example, ischemic hindlimbs [Proc Natl Acad Sci U S A 2008; 105: 14347-52] and areas of myocardial infarction [Science 1997; 275: 964-967]. Recently, EPCs have also been used to engineer blood vessels. It has been shown that an intramyocardial injection of autologous EPCs or intravenous administration of EPCs can increase vasculogenesis and improve cardiac function after myocardial infarction in animal experiments and clinical trials [Proc Natl Acad Sci U S A 2005; 102: 10999-11004]. However, abundant evidence suggests that EPCs contribute to vascular repair, remodeling, and lesion formation under physiological and pathological conditions. Systemic administration of EPCs also improves functions of ischemic tissues after stroke [Circ Res 2009; 104: 969-977] or myocardial infarction [Exp Hematol 2009; 37: 8-9]. Thus, it is hypothesized that homing and differentiation of EPCs largely depend on their signaling cascade that occurs upon artificial vascular graft.

Polyurethane (PU) is one of the most interesting synthetic elastomers [45]. Because of its unique properties, more attention has been paid to the synthesis, morphology, chemical, and mechanical properties of the polymer. PU is also widely used in biomedical applications because of its good biocompatibility and mechanical properties [Artif Organs 2000; 24: 119-128]. Gold (Au) is regarded as one of the noble metals with high biocompatibility. Au nanoparticles were used for immobilization of biomolecules such as proteins, enzymes, and antibodies [Biomaterials 2004; 25: 3445-3451]. This combination has been recently used as a biomimetic interface to construct a cellular biosensor [Biomaterials 2004; 25: 3445-3451]. The change in surface morphology of the PU in surface morphology upon addition of Au was more complicated than that of the earlier poly(ether urethane) system [J Biomed Mater Res A 2006; 79: 759-70]. The PEU-Au nanocomposites showed surface morphological difference only in the size of hard-segment micelles upon addition of Au nanoparticle [J Biomed Mater Res A 2006; 79: 759-70]. The other PSU-Au nanocomposites showed various distinct surface morphologies, ranging from hard-segment lamellae to soft-segment micelles [Biomacromolecules 2008; 9: 241-8]. It was suggested that the phase separation was probably stabilized by hydrogen bonds caused by the gold nanoparticless. The better improvement of in vitro and in vivo biocompatibility of this PU at such low Au concentrations (43.5 ppm) is believed to be a result of the altered surface morphology as well as associative effects in the presence of Au [Artif Organs 2000; 24: 119-128; J Biomed Mater Res A 2006; 79: 759-70; J Biomed Mater Res A. 2008 Mar 1;84(3):785-94; Biomaterials 2009; 30: 1502-11; Biomacromolecules 2008; 9: 241-8]. The surface morphological change in PU induced by Au nanoparticles could affect the migration of endothelial cells cultured on these nano-biomaterials, as well as the up-regulation of eNOS expression via PI3K/Akt signaling pathway has also been found in our recently report [Biomaterials. 2009 Mar;30(8):1502-11; N Biotechnol 2009; 25: 235-43].

The homing mechanisms that promote recruitment of EPCs are still unclear. EPCs are believed to migrate via the through an endothelial barrier, and be retained in bone marrow niches [J Biol Chem 279: 49430-49438; 2004; Blood 89: 72-80 , 997; 1997]. These processes are mediated by cell adhesion molecules and chemokines. Stromal-derived factor (SDF-1) is constantly secrected by bone marrow stromal cells, immature osteoblasts, and endothelial cells [Blood 89: 72-80, 1997]. Recent report evident for regulating mobilization of pro-angiogenic EPCs or mesenchymal stem cells (MSCs) in vivo has been indicated by elevation of SDF-1, which stimulates mobilization of CXCR4 integrin expression [Immunity 25: 977-988, 2; 2006; Stem Cells 24: 1254-1264; 2006]. These effects are abolished in the lake of tissue injury, suggesting that SDF-1/CXCR4 signals promoted by damaged tissue are required for the contemporary of EPCs in the vascular niche within tissues. However, the role of this SDF-1/CXCR4 to

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modulate the response of EPCs on the PU-Au nanocomposites is so far unknown.

The present study was mentioned to evaluate the effect of implantation of EPCs on regeneration of endothelium in damaged vessels. Because our previous reports have demonstrated that PU-Au nanocomposites could promote migration and proliferation effects on ECs [Biomaterials. 2009 Mar;30(8):1502-11], we hypothesized that implantation of EPCs on PU-Au nanocomposites may effectively useful for repairing of vascular tissue. Furthermore, existing evidence suggests that paracrine effect of EPCs due to secretion of growth factors may contribute to overall beneficial effect on endothelial repair [Circulation. 2003;107:1164 –1169; Circ Res. 2004;94:678 – 685]. To address this possibility, we also intended to explore the molecular mechanism to identify SDF-1α secreted by EPCs that may help explain their regenerative ability. We propose to elucidate the specific angiogenic processes downstream of SDF-1/CXCR4 receptor activated by PU-Au nanocomposites in the EPCs, and further to explore how critical role played by endothelialization after vascular injury, and to investigate possible therapeutic strategies by using PU-Au nanocomposites to modulate this contribution.

Materials & Methods

Polyurethane dispersion and the diisocyanate salt were supplied by Great Eastern Resins Industrial Co., Taiwan. The polyurethane dispersion (50% solid content in distilled water) was synthesized based on a molecular ratio 3:1 from hexamethylene diisocyanate (HDI) and the macrodiol poly(butadiene adipate), and further chain-extended by ethylene diamine sulfonate sodium salt and ethylene diamine. The diisocyanate salt was a mixture of isocyanurate trimer of hexamethylene diisocyanate (HDI trimer) and 6% Bayer hardener (made from HDI trimer and polyethylene glycol). The final polymer had a hard segment weight fraction of about 34.6% [7, 17]. Au nanoparticles (manufactured by Gold NanoTech, Taiwan) were supplied as a suspension (50 ppm/ml in distilled water). The diameter of the Au nanoparticles was uniform in the range of 4-7 nm (average 5 nm), as determined by transmission electron microscopy [Biomacromolecules 2008; 9: 241-8; Biomaterials. 2009 Mar;30(8):1502-11].

Preparation of polyurethane-gold (PU-Au) nanocomposite films

The polyurethane dispersion was diluted by distilled water or Au suspension to 10 wt% solid content. The diisocyanate salt was added to the polyurethane dispersion (concentration of the diisocyanate salt at 1 wt%), and the mixture was stirred for 30 min to obtain the suspension of plain polyurethane (denoted “PU”) or polyurethane-gold (denoted “PU-Au”) nanocomposites. The PU-Au suspensions were prepared to contain 43.5 or 174 ppm of Au in the final nanocomposites after water removal. Thin films (~0.02 mm) were cast from the PU or PU-Au suspension on 15-mm (Superior, Germany) or 32-mm round glass coverslips (Assistant, Germany) by a spin coater (Synrex Pm-490, Taiwan). They were dried at 60^{o}C for 48 h and further dried in a vacuum oven at 60^{o}C for 72 h to remove any residual solvent [7, 17]. Their surface morphology and microphase separation were confirmed by atomic force microscopy [Biomacromolecules 2008; 9: 241-8; Biomaterials. 2009 Mar;30(8):1502-11].

Methods

2.1. Adult peripheral samples and buffy coat cell preparation

The approval of the local ethical committee for this study was obtained. To collect endothelial progenitor cells (EPCs), the blood sample was taken from blood sample with young health adults. Peripheral blood mononuclear cells (PBMC) were obtained using a modified density gradient technique. Briefly, blood sample (20~100 ml) was diluted 1:1 with Hanks balanced salt solution (HBSS; Invitrogen, Grand Island, NY) and was overlaid onto an equivalent volume of Ficoll isopaque (Sigma, USA). Peripheral blood mononuclear cells (PBMCs) were centrifuged for 30 minutes at room temperature at 1500 rpm. MNCs were isolated and washed 3 times with RPMI medium (Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT), 2% penicillin/streptomycin (Invitrogen, USA).

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2.2. Culture of adult peripheral blood endothelial cells

MNCs were resuspended in 12 mL complete RPMI medium supplemented with 50 ng/ml of vascular growth factor (VEGF) (Sigma, USA) (completed medium). Cells were seeded onto 3 separate wells of a 6-well tissue culture plate precoated with fibronectin (Sigma, USA) at 37°C, 5% CO_2, in a humidified incubator. After 24 hours of culture, nonadherent cells and debris were aspirated, adherent cells were washed once with complete medium was added to each well. Medium was changed daily for 7 days and then every other day until the first passage. Endothelial progenitor cells (EPCs) appeared between 5 and 22 days of culture and were identified as well-circumscribed monolayers of cobblestone appearing cells. EPCs were enumerated by visual inspection using an inverted microscope (Olympus, Lake Success, NY) under 40 × magnification.

2.3. Fluorescence-activated cell sorter (FACS) analysis for CD34

In order to determine the percentage of $CD34^+$ cells in PBMCs, the PBMCs fraction was incubated with 10 µl of FITC-labelled anti CD34 MAb (Miltenyi Biotec Ltd, Bisley, Surrey, UK) for 10 min. PBMC also were incubated with a control phycoerythrin-labelled mouse IgG1 and FITC-labeled mouse IgG2a. Two-color flow cytometric analyses were performed by FACS scan. Each analysis included more than 10,000 events.

2.4. Proliferation assay

The MNCs were cultured in M199 medium (Gibco, USA) supplemented with 10% FBS, 50 ng/ml vascular endothelial growth factor (VEGF) (Sigma, USA) in the presence of penicillin (100 Units/ml), streptomycin (100 mg/ml) as basic medium was used in this study. PU and PU-Au nanocomposites on 15-mm coverslips were sterilized by 70% ethanol, rinsed and placed into the bottom of 24-well tissue culture plates (Corning, USA). One ml of cell suspension containing 2×10^4 cells was injected into each well of the culture plates. Cells cultured in a blank well (tissue culture polystyrene, TCPS) were used as control. After day1 and day 3 of incubation, the adherent cells were harvested for MTT assay. Briefly, 3-(4,5)-dimethylthiahiazo (-z-y1)-3,5-di-phenyltetrazolium bromide (MTT) solution [(0.5 mg/ml, 1× phosphate buffered saline (PBS)] was added and incubated for 4 h at 37°C. The supernatant was removed and the aliquot dimethyl sulfoxide (DMSO) (Tedia, Fairfield, OH, USA) was added into each well to dissolve any resulting formazan crystals for 10 min. The absorbance was measured at 550 nm with an ELISA reader (F-2500, Hitachi, Japan).

2.5. Immunofluorescence staining

Cells (2×10^4 cells per well) were seeded in 24-well plates with material-coated coverslips and incubated. After 48 h in culture, they were fixed with 4 % paraformaldehyde and permeabilized with 0.5% (v/v) Triton X-100 in PBS for 10 min prior to staining. Following fixation and permeabilization, non-specific binding was blocked by adding 1% (w/v) BSA for 30 min at room temperature. Cells were incubated in the primary anti-eNOS antibody solution (1: 300 dilution, Santa Cruz, USA) (for BAEC) and primary anti-vWF antibody solution (1:300 dilution, Santa Cruz, USA) for 60 min, washed extensively and then incubated with the appropriate secondary Cy5.5-conjugated immunoglobulin (red color fluorescence: anti-eNOS antibody) (1: 300 dilution) or FITC-conjugated antibody (green color fluorescence: anti-vWF antibody) (1: 300 dilution) for 60 min. Following further extensive washing, the nuclei were stained with 4', 6'-diamidino-2-phenylindole (DAPI) (1: 500 dilution) for 20 min. the cells were stained with rhodamine phalloidin (1: 1000, Sigma) for 30 min. After further washes, coverslips were mounted on microscope slides with the storage solution (glycerol/PBS) and sealed with a synthetic mount. In each case a primary antibody free control was produced. Images were collected on fluorescence microscope.

2.6. Fluorescent labeling of cytoskeletal fibers

EPCs were seeded in 24-well plates with coverslips at a density of 0.5×10^4 cells per well and incubated under standard conditions for 8 hr. After incubation, the cells were washed with PBS buffer, fixed with 4% paraformaldehyde for 15 min, and

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permeabilized with 0.5% (v/v) Triton X-100 in PBS for 10 min prior to staining. Following fixation and permeabilization, non-specific binding was blocked by adding 1% (w/v) BSA for 30 min at room temperature. Following further extensive washing, the cells were stained with rhodamine phalloidin (1:1000, Sigma) for 30 min. After three additional washes, the coverslips were mounted on microscope slides and sealed with a synthetic mount. Images were collected on the fluorescence microscope (Eclipse 80i, Nikon, Japan).

2.7. Flow cytometric of surface marker analysis

Cells were seeded into each well of the 6-well tissue culture plate containing material-coated coverslips as described. After 48 h of incubation, the cells were collected by trypinization. The cells were detached with 2 mM EDTA in PBS, washed with PBS containing 2% BSA and 0.1% sodium azide (Sigma, USA) and incubated with the respective antibody conjugated with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) against the indicated markers: CD29-PE, CD34-FITC, CD44-FITC, CD31-PE, CD106-FITC and CD105-PE, CD166-PE and VEGF-R2-PE (BD, Pharmingen, USA). PE-conjugated IgG1 and FITC-conjugated IgG1 were used as isotype controls (BD Pharmingen, USA). Thereafter, the cells were analyzed by a FACS calibur flow cytometer (Becton Dickinson, USA). Cells were then treated with phycoerythrin or FITC-goat anti-mouse immunoglobulin antibody, washed three times in PBS and then analyzed by a FACScalibur flow cytometer (Becton Dickinson, USA). Fluorescein-positive cells were processed using the WinMidi software.

2.8. Reverse transcription polymerase chain reaction (RT-PCR) analysis

2×10^5 cells in 5 ml of the culture medium were seeded into each well of the 6-well tissue culture plates containing 32 mm material-coated coverslips. Cells were then incubated at standard conditions. After 48 h of incubation, the adherent cells were trypsinized with 0.025% trypsin-EDTA and the total RNA was extracted by TRIZOL® reagent (Invitrogen, 15596-018, India) by following the manufacturer's instructions. 0.5 μg of total RNA was reverse transcribed into cDNA by using random hexameric primers (0.5 μg) and AMV reverse transcriptase (Reverse Transcription System, Promega, USA). Aliquots of the cDNA products were used as templates for PCR amplification in a thermal cycler. Specific primers for eNOS, forward primer: 5′-ATA-GAA-TTC-ACC-AGC-ACC-TTT-GGG-AAT-GGC-GAT-3′, reverse primer: 3′-ATA-GAA-TTC-GGA-TTC-ACT-GTC-TGT-GTT-GCT-GGA-CTC-CTT-3′, product size: 198 bp; GAPDH forward primer: 5′-TCC-CTC-AAG-ATT-GTC-AGC-AA-3, reverse primer: 5′-AGA-TCC-ACA-ACG-GAT-ACA-TT-3′, product size: 198 bp. 94°C 5 min (1 cycle), 94°C 30 sec, 56°C 30 sec, 72°C 45 sec (28 cycles), 72°C 7 min (2 cycles) with GeneAmp® PCR System 2700 (Applied Biosystems, US). Control reactions were performed in the absence of cDNA. PCR products were separated on 2% agarose gels by electrophoresis and visualized by staining with ethidium bromide. Images were acquired by LabWork Image Acquisition and Analysis software (BioDoc-It™ System; Ultraviolet Products, USA) and expressed as the eNOS/GAPDH ratio. The tests were performed three times and the representative data were shown.

2.9. Surface grafting of PU-Au

Gold (Au) nanoparticles (Global NanoTech, Taiwan, 180 ppm pure 5 nm Au fine particles in water) were mixed with the filtered polyurethane (PU) solution. The final Au concentration in the polymer was 43.5 ppm [Biomaterials 2009; 30: 1502-11]. The plasma surface of catheter coated with polyurethane-gold nanocomposites (PU-Au) was grafted by air plasma equipment. The plasma equipment used was an open air plasma system (Openair®) developed by Plasmatreat (Steinhagen, Germany). The apparatus included a power generator, a transformer of high voltage power supply and a rotating nozzle. The operating platform was designed and assembled by the San Fan Machinery Company (Taipei, Taiwan). The plasma source was compressed dried air (21% oxygen and 79% nitrogen). Air plasma was ejected from a rotating nozzle. The temperature of the plasma at the exit of nozzle was 65°C. The pressure and power of the air plasma as well as the distance and scan speed of the plasma nozzle were among the parameters that could be adjusted. For the current experiment, parameters were optimized as follows. The air pressure was 2.5 kg

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cm^{-2}. The plasma power was set at 1000 W. The substrate was placed at a distance of 10 mm from the nozzle. The scan speed of the nozzle was 15 m min^{-1}. Immediately after the plasma treatment, the substrates were immersed in PU solution containing an extra 43.5 ppm of Au) at 37°C for 1 h. The substrates were then rinsed with distilled water, washed extensively in an ultrasonic bath for 30 min, air-dried and stored in a desiccator. The process of modification is illustrated in Fig. 6. All substrates were washed and dried by the method described.

2.10. Cell seeding and maintenance of materials in vitro

First, cells (1×10^6 cells/ml) were suspended in 1 ml culture medium and label with Quantum Dot (Invitrogen, USA), a fluorescent dye that bind the cell membrane, at a concentration of 10 nM. The labeling procedure was performed according to the manufacturer's protocol with some modifications to optimize the procedure for cells. PU-Au materials with cells were rolled into catheter with the 3D shaker (Gene Pure, GDS100-1) for 48 hr. After rolling, materials were checked for their attachment ability under fluorescence microscopy before implantation.

2.11. Implantation of cell-seeded materials

Female New Zealand White rabbits (2.5 to 3 kg) were used in all experiments. All materials (1×10^6 cells per material) were implanted into the femoral artery of rabbit. Three experimental groups were studied for cells implantation with: (i) control (without treatment) (ii) treatment with catheter (iii) PU-Au coated with catheter, (iv) PU-Au plus cells coated with catheter. Cells-seeded vascular catheters were implanted into the femoral arteries of rabbit. All tissue constructs were harvested at 4 weeks after implantation for analyses. The abbit were anesthetized with intramuscular injection of ketamine (30 mg/kg) and intravenous injection of pentobarbital (30 mg/kg) and ventilated with a mixture of O_2, N_2 and isoflurane during the operation. The femoral artery was exposed and the surrounding tissues were dissected from the artery. After heparin (100 unit/kg) was given intravenously, the proximal and distal portions of the femoral artery were clamped. The femoral artery was clamped and ligated, and the graft was placed end to-end and sutured with 10-0 interrupted stitches. A 40 mm length of the femoral artery was dissected, and the vessel was washed with a heparin solution and transplanted with the vascular grafts (catheter) using a 22 GA1 (BD, USA). No anti-coagulants or anti-platelets were administered post-operatively. The graft was removed by ligation of native femoral artery directly adjacent to the suture locations. All animals received humane care in compliance with the ''Guide for the Care and Use of Laboratory Animals'' published by the National Institutes of Health.

2.12. Histological and immunohistochemical examination

We took 3 sites sample from each implanted grafts for further analysis, the distal part, proximal part and the middle part. The distal and proximal part included the interface between the engineered tissue and native tissue. Segments of the blood vessels harvested 4 weeks after implantation were fixed in 10% buffered formaldehyde solution, dehydrated with a graded ethanol series, and embedded in paraffin. The specimens were cut into 4 mm-thick sections and stained with hematoxylin and eosin (H&E). Immunohistochemical staining was used to analyze the sections with the following primary antibodies: anti-CD31 antibody (DAKO, USA), and anti-CD34 antibody (DAKO, USA) along with FITC-IgG secondary antibody and double confirmed following staining with 3,3'-Diaminobenzidine (DAB) (DAKO, USA). Native femoral artery from rabbits served as positive controls for all stains. Immunohistochemistry images were captured with a fluorescence microscopy (Zeiss Axio Imager A1, USA).

2.13. Statistical analysis

Multiple samples were collected in each measurement and expressed as mean ± standard deviation. Single-factor analysis of variance (ANOVA) method was used to assess the statistical significance of the results. p values less than 0.05 are considered significant.

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<Results>:

Characterization of the nanocomposites and endothelial progenitor cells

The AFM images was shown that the presence of Au nanoparticle at the concentration of 43.5 ppm induced a surface morphology change from the more aggregated hard-segments of original PU to the nanopattern with homogenous hard and soft domains, as evident in the Figure 1A. Peripheral blood mononuclear cells (PBMCs) (2×10^6 cells) were obtained from 20 ml of venous blood (Fig 1B). Flow cytometry assay showed that about 1.09% of these cells expressed CD34 surface marker. A representative analysis is shown in Figure 1C. Endothelial progenitor cells (EPCs) are well characterized as a CD133 surface marker subpopulation of CD34-positive cell. At 1, 7, and 14 days of incubation, the morphology of EPCs was visualized by microscopy. The defined cells morphology exhibited as a central core of round cells in day1 and exhibited with elongated cells at the periphery was shown from the day 7 to day 14 (Figure 1D). Indeed, cells were also identified by staining for endothelial lineage markers as shown in Figure 1E. The endothelial cells phenotype was characterized by immunostaining with antibodies specific for endothelial markers (vWF and eNOS) at day 21.

Cell proliferation and adhesion assay

The viability assay of EPCs that not proliferated on PU-Au nanocomposites after day 1 and day 3 incubation is shown in Figure 2A. However, the numbers of EPCs on PU-Au was shown had better attachment ability than on the original PU. This is also observed by the slightly up-regulated the CD29 surface marker by PU-Au nanocomposites. However, this effect was less obvious observation of CD44 surface marker. Furthermore, PU-Au had the better actin fiber expression, followed by FN, PU and TCPS (Figure 2C). This result agreed with the enhanced adhesion of ECs on PU-Au observed earlier.

eNOS protein expression was induced by PU-Au nanocompoosites

The eNOS mRNA expression at day 7 and day 14 was induced by PU-Au nanocomposites, as shown in Figure 3A. EPCs showed a lower level of eNOS gene expression on TCPS, FN or PU than on PU-Au nanocomposites. The expression and cellular localization of eNOS for EPCs cultured on the materials for day 7 and day 14 also confirmed by immunofluorescence. As evident from Figure 3B, PU-Au promoted the more homogenous localization of eNOS. An increase of fluorescence intensity in the cytoplasmic distribution of eNOS for EPCs cultured on PU-Au was also demonstrated through the image analysis.

PU-Au nanocomposites promoted the maturation of EPCs partially through the SDF-1/CXCR4 pathway

PU-Au nanocomposites significantly promoted CD31, CD34 and VEGF-R2 expression after SDF-1α treatment for day 14 compared to without treatment group was observed in Figure 4A. We further characterized the SDF-1/CXCR4 signaling of EPCs on PU-Au nanocomposites. It was noticed that the CD31, CD34 and VEGF-R2 expression level was blocked by CXCR4-antibody and AMD3100. Indeed, the reduction effect of CD34 on PU-Au was exhibited more prominent than that on other surface markers (Figure 4B). Since EPCs showed the distinct feature of EPCs differentiation, a comparison was analyzed the hematopoietic properties relating to their expression of surface markers. Despite the clear differentiate in endothelial cells in EPCs on PU-Au nanocompomsites, EPCs did not exhibited a similar other cell surface markers expression profile on EPCs on PU-Au. As shown in Figure 5, EPCs was negative for hemoatopoietic (CD34, CD45) and endothelial (CD105, cd106, CD166) cells.

PU-Au nanocomposites promote the endothelialization in the vascular disease model

The EPCs-seeded materials of rabbit femoral artery divided in 3 groups (catheter treated, PU-Au with catheter, PU-Au plus PECs with catheter). To determine the graft potency, cells adhesion ability was tested by using fluorescence tracer test before implantation. As shown in Figure 6 B, EPCs labeled with fluorescent QD prior to implantation on PU-Au were exhibited better attachment ability than control group (without coated with PU-Au). The procedure of implantation cell-seeded materials as depicted in Figure 6C.

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Antithrombogenic property of EPCs after coated with PU-Au nanocomposites

To clarify the repairing capacity of long-term biomimetic remodeling of cellular vascular grafts coated with EPCs on PU-Au nanocomosites, Next, we furthermore performed long-term experiments to examine the vascular lumen of cellular grafts. For vascular graft, it is necessary to use biomaterials that are biocompatibility and biominic to the etracellular matrix (ECM) property in native vascular tissue. To determine the interactions of EPCs with PU-Au, in vivo experiment was performed by seeding EPCs on PU-Au. EPCs seeded with PU-Au nanocomposites had better cellular organization and proliferation effect similar to that of the native vascular tissue (blank) (Figure 7A). It was suggested that PU-Au mimicked native endothelial ECM matrix and guided EPCs more readily differentiate to endothelial cells. Because thrombosis is the major high risk factor of vascular grafts, especially for small diameter of vascular, we carried out more detailed analysis on platelet adhesion and thromous formation. HE staining assay revealed that acellular graft (with control group) had significant amounts of platelet aggregation after 28 day implantation (Figure 7B). However, the EPCs grafts exhibited very little platelet aggregation on the lumen surface, which coated with the PU-Au nanocomposites, followed by PU-Au (without implantation of EPCs) treatment group. Cross-section staining showed thrombus formation on the luminal surface of acellular grafts (with treatment) but not of EPCs-implantation grafts. Platelet aggregation and thrombus are an index of host response to foreign-body biomaterials; our results demonstrated that when seeded EPCs with PU-Au nanocomposites could have better antithrombogenic capacity. Consistently, the results at 1 month significant intimal thickening in acellular grafts but not in EPCs-seeded grafts (Fig. 7B). Statistical analysis demonstrated that the lumen area of the cross-sections blocked by intimal tissue was significantly higher in acellular grafts after 1 month implantation. It is conceivable that the long-term patency of EPCs-MSC-seeded vascular grafts (coated with PU-Au nanocomposites) was mainly because of the antithrombogenic property of EPCs in the early phase.

EPCs promoted vascular regeneration by PU-Au nanocomposites

Immunohistochemical examination of vascular graft coated with PU-Au nancomposites after1 month post-implantation of EPCs showed regeneration of vascular tissues of the artery on endothelial layer. Immunohistochemical analysis showed that cells on the luminal sides (endothelial layer) of the vascular grafts stained significantly stained positively expression for CD31 and CD34, further indicating the endothelium regeneration (Fig 8).

<Discussion>

Tissue engineered vascular conduits need to have the mechanical and biological properties of arteries in order to have superior patency rates. The conduits need to be non-thrombogenic and need to simulate arteries. The capacity of peripheral blood mononuclear cells (PBMCs) derived endothelial progenitor cells (EPCs) has important implications in the repair of vascular tissue. Reports also suggested that EPCs are derived from PBMCs and that accumulate at sites of angiogenesis [J Surg Res 2005; 126: 193-8]. These studies support the notion that EPCs could be a cell source for ECs and would be an ideal cell source for tissue engineering of autologous vascular grafts [Circulation 2002; 106: 1913-8]. An ideal strategy is to prepare a material which is inherently nonthrombogenic, compliant and has the ability to promote endothelialisation from endothelial progenitor cells (EPCs) directly from the blood [Circulation 2002; 106: 1913-8]. So far, anti-thrombotic modifications of the intima of vascular grafts can inhibit thrombosis, but these anticoagulant effects are temporary, and vascular grafts are meant to last for the life of the patient [Biomaterials 2009; 30: 79-88]. Since EPCs play important roles in thrombosis, endothelialization of the vascular graft is the most effective way to deal with thrombosis and intima hyperplasia [Expert Rev Cardiovasc Ther 2008; 6: 1259-77]. Based on our current research, we took peripheral mononuclear cells and isolated the EPCs from the monocytes by changing the culture medium after the first day. Using a combination of surface marker discrimination and adhesion-based EPC isolation, we developed a new PU-Au nanocompsites to explore the vascular tissue research.

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Mobilization from the bone marrow can increase the local number of EPCs; stromal derived factor-1 α (SDF-1α), it can mobilize EPCs to promote the formation of new blood vessels [Circulation. 2010 Sep 14;122(11 Suppl):S107-17]. Our results showed that PU-Au can improve the adhesion ability of circulation EPCs effectively. In particular, it can significantly enhance the mobilization function of SDF-1α/CXCR4, indicating that PU-Au can promote EPCs mobilization alone and synergy with VEGF. Indeed, we also demonstrate that PU-Au mobilized EPCs by increasing the levels of CD34 surface marker expression possibility through the SDF-1α/CXCR4 signaling pathway. PU-Au induced differentiation and endothelialization of EPCs occurs through CXCR/SDF-1α signaling pathway has been observed in our current study. Because the function of SDF-1α/CXCR4 is very diversity, further studies are necessary to determine the signal mechanism in different process. Based on these finding, the specific mechanisms (ex: VEGF) of cross-talk between the vascular tissue and PU-Au nanocomposites in EPCs deserve further exploration. EPCs can be mobilized from bone marrow to peripheral blood, homing to the damages site will repair the injured blood vessel [Science 1997; 275: 964-967; Circulation 2002; 106: 1913-8]. Our results indicate that PU-Au can promote EPCs differentiation into endothelial cells that expression the endothelia-special markers CD31 and VEGF-R2. Based on these finding, it was suggested that PU-Au can promote EPCs mobilization and homing to promote endothelium of vascular graft. To test this concept, we prepared a "commercial catheter", coated PU-Au nanocomposites on the surface and seed EPCs on the "vascular graft", and then transplanted the vascular graft in the rabbit femoral artery model.

During the implantation of vascular stents, percutaneous transluminal coronary angioplasty (PTCA) tends to damage the vascular endothelium [Tissue Eng 2005; 11: 1574-1588]. If EPCs can be mobilized from bone marrow to peripheral blood, homing to the damages site will repair the injured blood vessel [Mol. Ther 2007; 15: 467-480]. Based on these finding, it was suggested that PU-Au can promote EPCs mobilization and homing to promote endothelium of vascular graft. To test this concept, we prepared a "commercial catheter", coated PU-Au nanocomposites on the surface and seed EPCs on the "vascular graft", and then transplanted the vascular graft in the rabbit femoral artery model.

The present results showed that the degree of endothelialization in PU-Au seeded with EPCs was significantly higher than the control vascular graft, consistent with the conclusion that PU-Au promotes CD34 and CD31 expression level of EPCs in vivo. Previously, the surface morphological change in PU-Au nanocomposites could promote the migration rate of ECs [Biomaterials 2009; 30: 1502-11]. The greater migration rate was associated with focal adhesion kinase (FAK) activation, cytoskeleton reorganization, as well as the modeulation of eNOS expression through the PI3K/Akt signaling pathway has been observed [Biomaterials 2009; 30: 1502-11; JBMRA, 2011, In press]. Indeed, our results also indicate that PU-Au can promote the eNOS protein expression in vitro. Although PU-Au can promote the repair of vascular tissue, it is unclear whether it can promote vascular growth modulated by eNOS. The potential effect of eNOS in PU-Au need further study.

Our results demonstrate that PU-Au nanocomposites allow the remodeling of vascular tissue in EPCs, similar to that the native vascular tissue. The effect of the antithrombogenic property of EPCs has also observed in this study. In this study we show that the PU-Au nanocomosites coated with the catheter allowed efficient promote endothelialization and that EPCs remodeling into lumen in the vascular wall, as in the native wall. The combination of antithrombic and implantation EPCs into PU-Au nanocomposites is a promising approach to fabricate ideal vascular grafts (e,g. allogenetic or autologus) that may be offer a new way for applied clinical setting. Although PU-Au had significantly induced of CD34 surface markers expression has been found in this study, however, it is still not obvious how much EPCs to the cell recruitment process from the host tissue. One important observation is the CD34 surface markers of vascular graft was continuous present in the vascular wall after 1 month implantation with PU-Au nanocomopsites. The relative contribution to endothelialization by the surrounding vascular and the circulating ECPs needs further investigation.

Given the ECPs from the vascular niche for endothelial cell differentiation and that vascular grafts are compatible with host vascular tissue in circulation. It was suggested that PU-Au nanocomposites can be a good model system for implantation of EPCs and to promote the endothelialization capacity after vascular graft implantation. Besides, our data also demonstrate that the antithrombosis property of EPCs depends on PU-Au nanocomposites, which provides a novel strategy for the approach of

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modifying vascular graft with antplatelet adhesion/aggregation effects. The use of ECPs which was isolation from peripheral blood monocyte cells to construct vascular graft by PU-Au nanocomposites could be a new insight for vascular tissue engineering. However, besides having an antithrombogenic effect on EPCs on PU-Au nanocomposites, ECPs could participate in other vascular repairing processes at the postimplantation, such as paprcrine signaling (ex: SDF-1 α/CXCR4) or ectracellular matrix (ECM) remodeling, which also needs warrants further studies.

Noteworthy, administration of EPCs into subjects with vascular disease has had lower proliferative efficacy with regard new vessel formation, which limited their clinical application [References]. To solve this problem, how to seek for other sources of hemoatopoietic stem cells for the better proliferative property will be worth exploration in the further. This is also supported by the significantly up-regulated expression of CD34 surface marker by PU-Au nanocomposites both in vitro and in vivo. Therefore, implantation of EPCs with PU-Au nanocomposites was shown to enhance in vivo endothelialization and the subsequent expression the CD34 marker in vascular damaged model. We further show that the CD34 surface marker of EPCs from PBMNCs can efficiency significantly induced by PU-Au nanocomposites under in vivo condition. The potential of CD34 surface marker to access recruit circulation EPCs from the host tissue and to differentiate into endothelial cells could be greatly beneficial for vascular tissue engineering application.

In this study, we show that the CD34 surface marker of EPCs from peripheral blood MNCs (PBMC) can efficiency significantly induced by PU-Au nanocomposites under in vivo condition. The potential of CD34 surface marker to access recruit circulation EPCs from the host tissue and to differentiate into endothelial cells could be greatly beneficial for vascular tissue engineering application. The use of more abundant PBMCs (ex: $CD14^+$ cells), could may offer other way to overcome limitation in cell number of EPCs. Because EPCs express functional SDF-1α/CXCR4 and interaction between eNOS and VEGF signaling pathway, it may form a positive-feedback loop to further enhance therapeutic neovascularization in vivo. The present study might have significant clinical implications in that induced CD34 surface markers by PU-Au nanocomposites has therapeutic potential for enhancing ischemia-induced neovascualrization, at least in part, by mobilizing EPCs to ischemic vascular tissue via the SDF-1 signaling pathway.

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Fig. 1

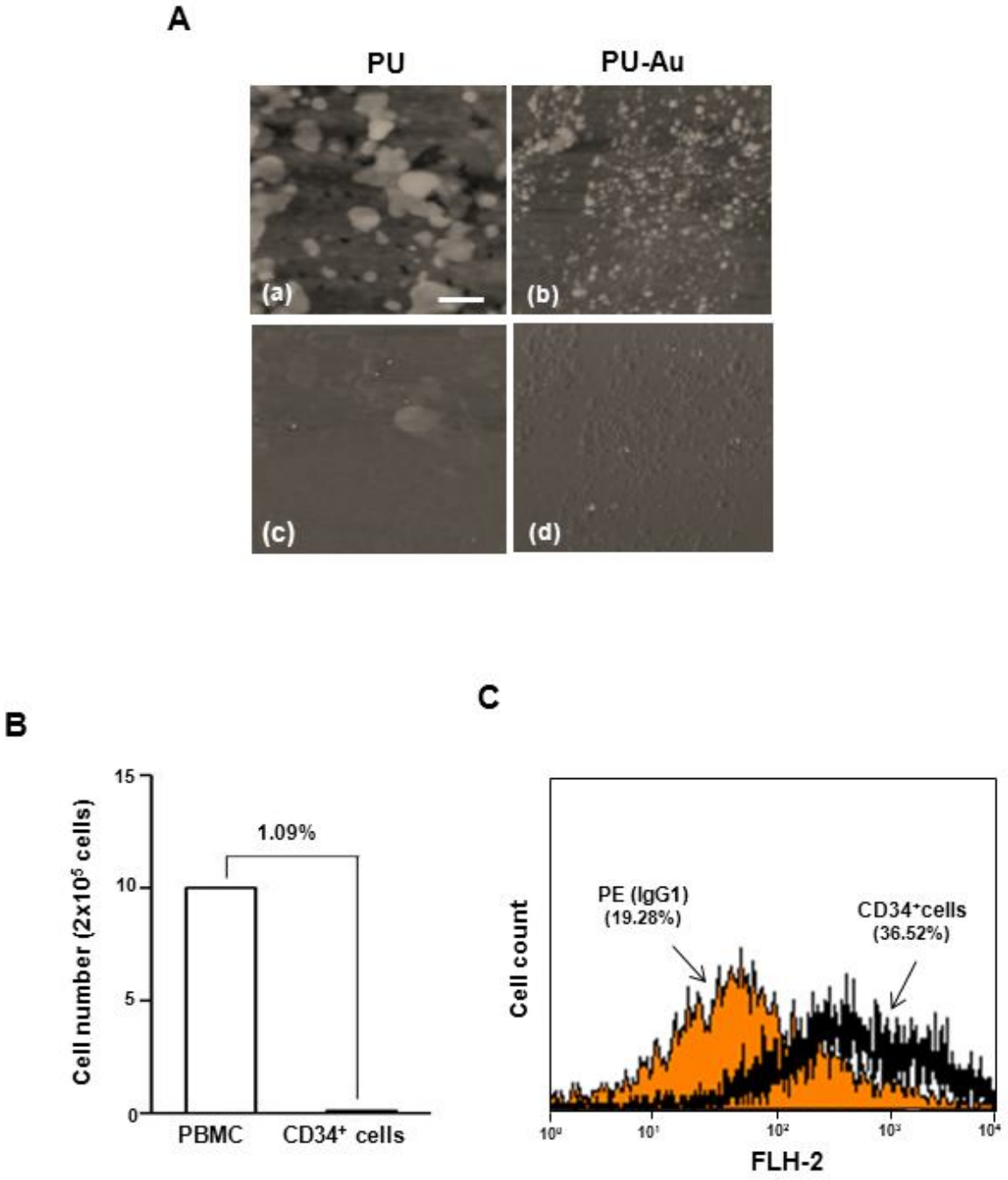

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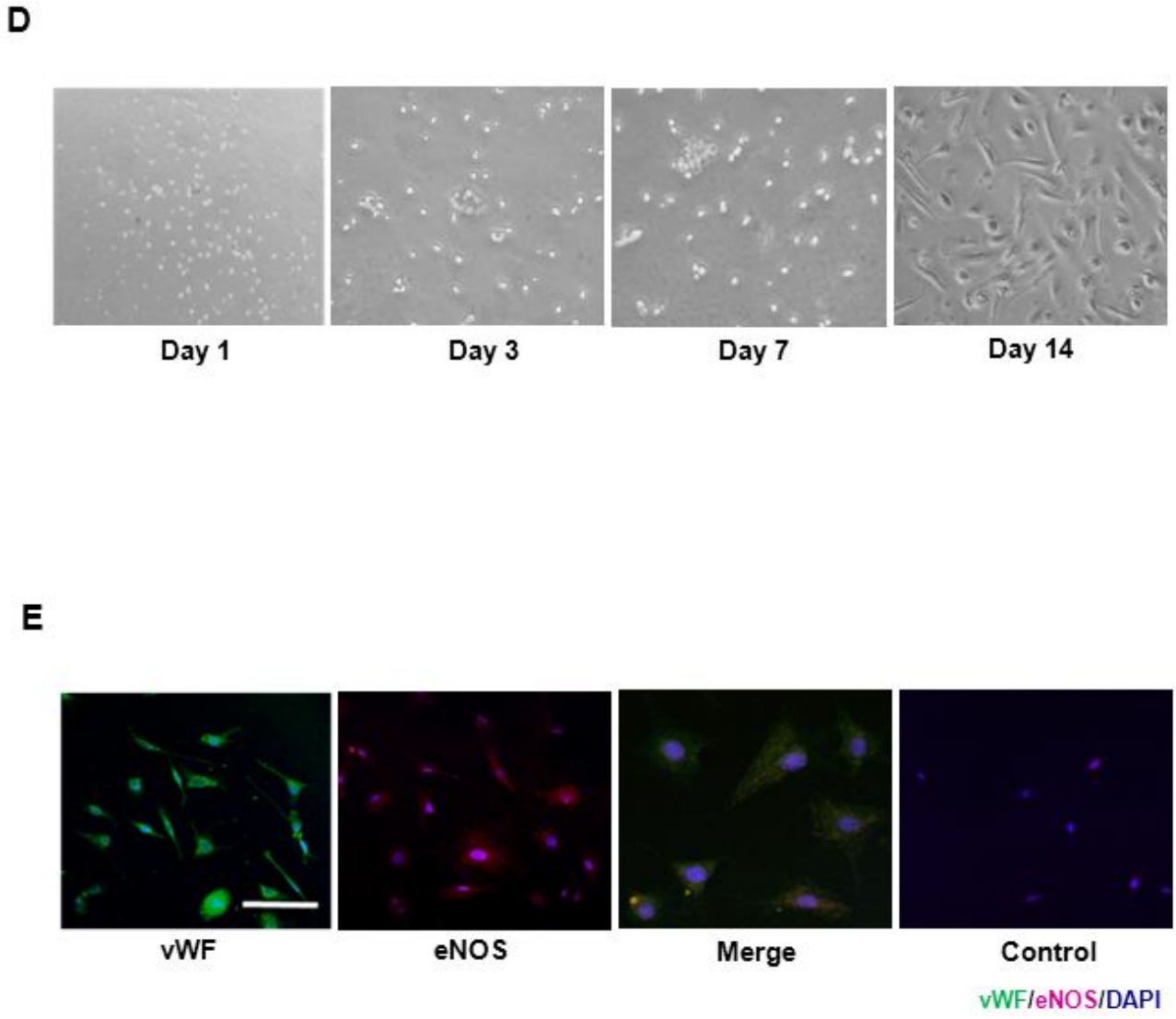
Fig. 1



<Figure 1>: **(A).** The AFM images for PU and the PU-Au nanocomposites containing 43.5 ppm of gold nanoparticles. Scale bar = 50 nm. **(B).** Small proportion of PBMCs and (C) expressed CD34 surface marker determined by fluorescence-activated cell sorter (FACS) analysis. **D.** Phase-contrast micrograph of endothelial progenitor cells (EPCs) colony. Scale bar = 100 μm. **(E).** The endothelial phenotype was confirmed by immunostaining with antibodies specific for endothelial markers: eNOS on cell membrane and on cells border to border (indicated by red color) and vWF contained within cytoplasmic granule (indicated by green color). Representative control sections where nuclear staining alone is visible. All cells were counterstained with DAPI showing the nucleus as blue.

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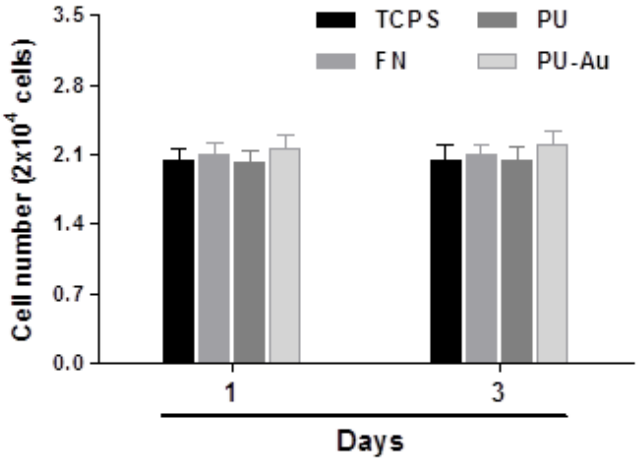
Fig. 2

A



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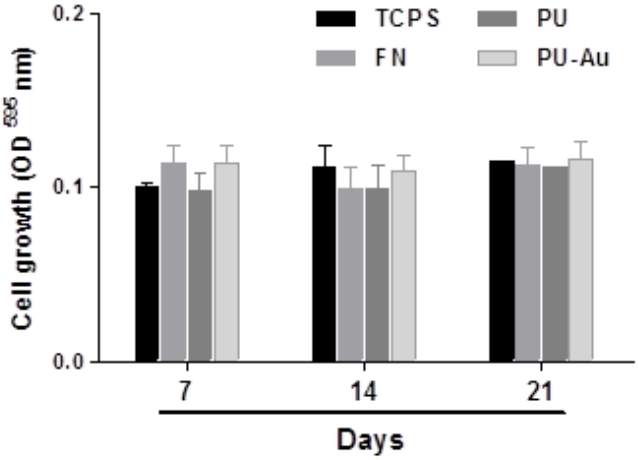

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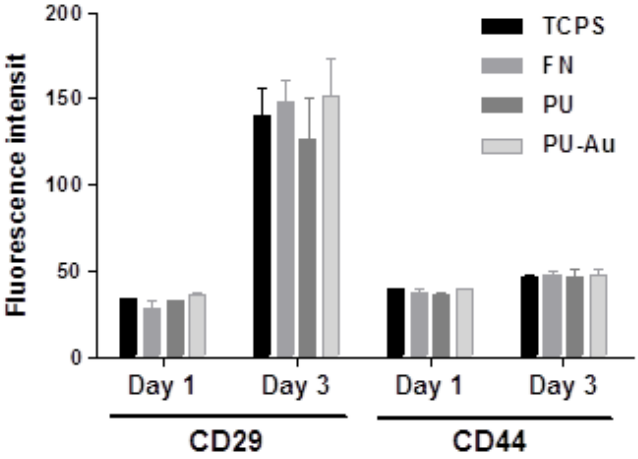

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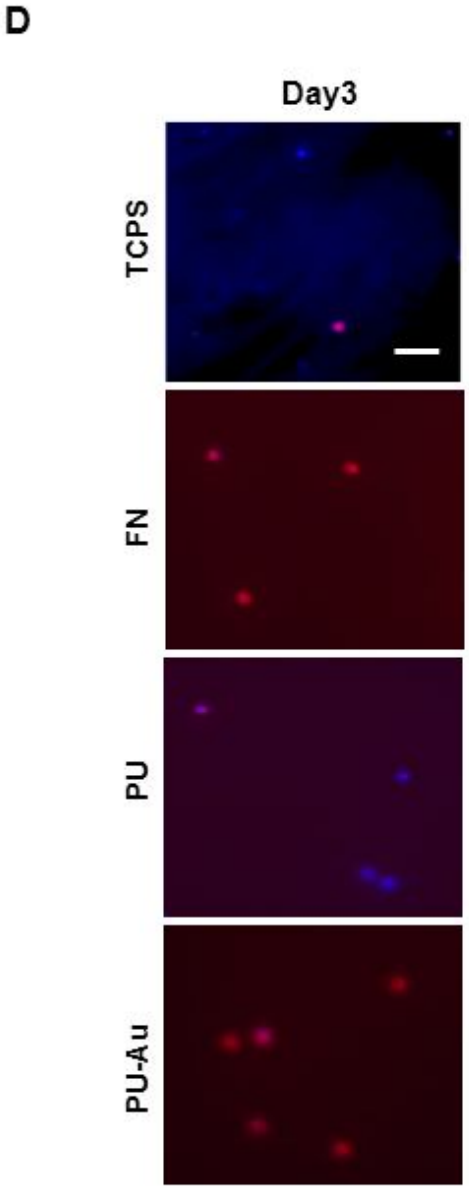
Fig. 2



<**Figure 2**> **:** Cell viability assay of cells cultured on four groups (TCPS, fibronectin, PU and PU-Au) on day 1 and day 3. Absorbance was measured in absorbance at 570 nm wavelength. **(B)**. Cell adhesion ability of endothelial progenitor cells (EPCs) on each materials for day 1 and day 3. Data are mean ± SD. **(C).** FACS analysis of CD29 and CD44 with materials for day 1 and day 3 incubation. Semi-quantitative measurement of EPCs cultured on each materials was showed. Mean ±SD. **(D**). The actin fiber distribution was confirmed by phalloidin staining. Scale bar = 100 μm.

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Fig. 3

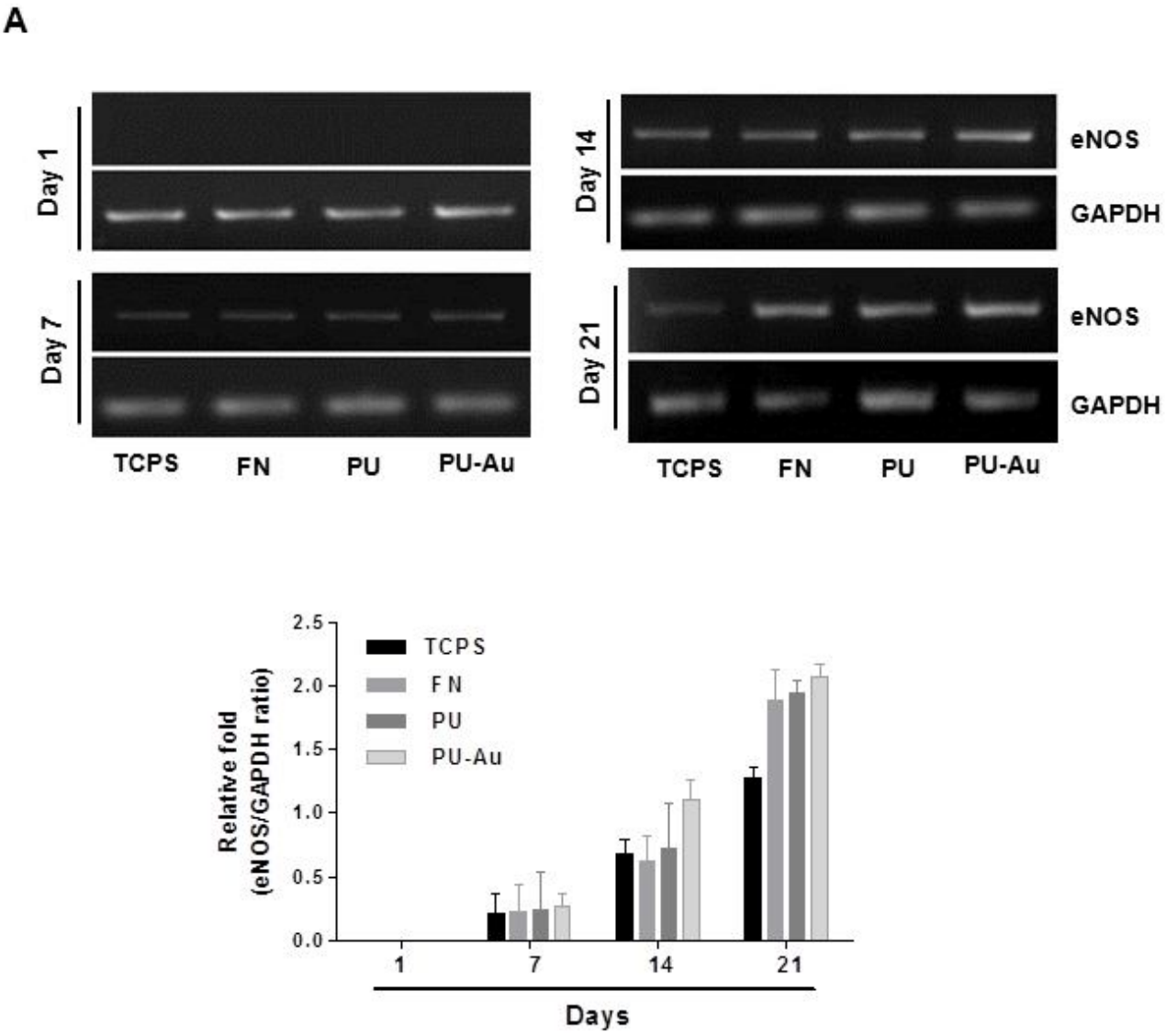

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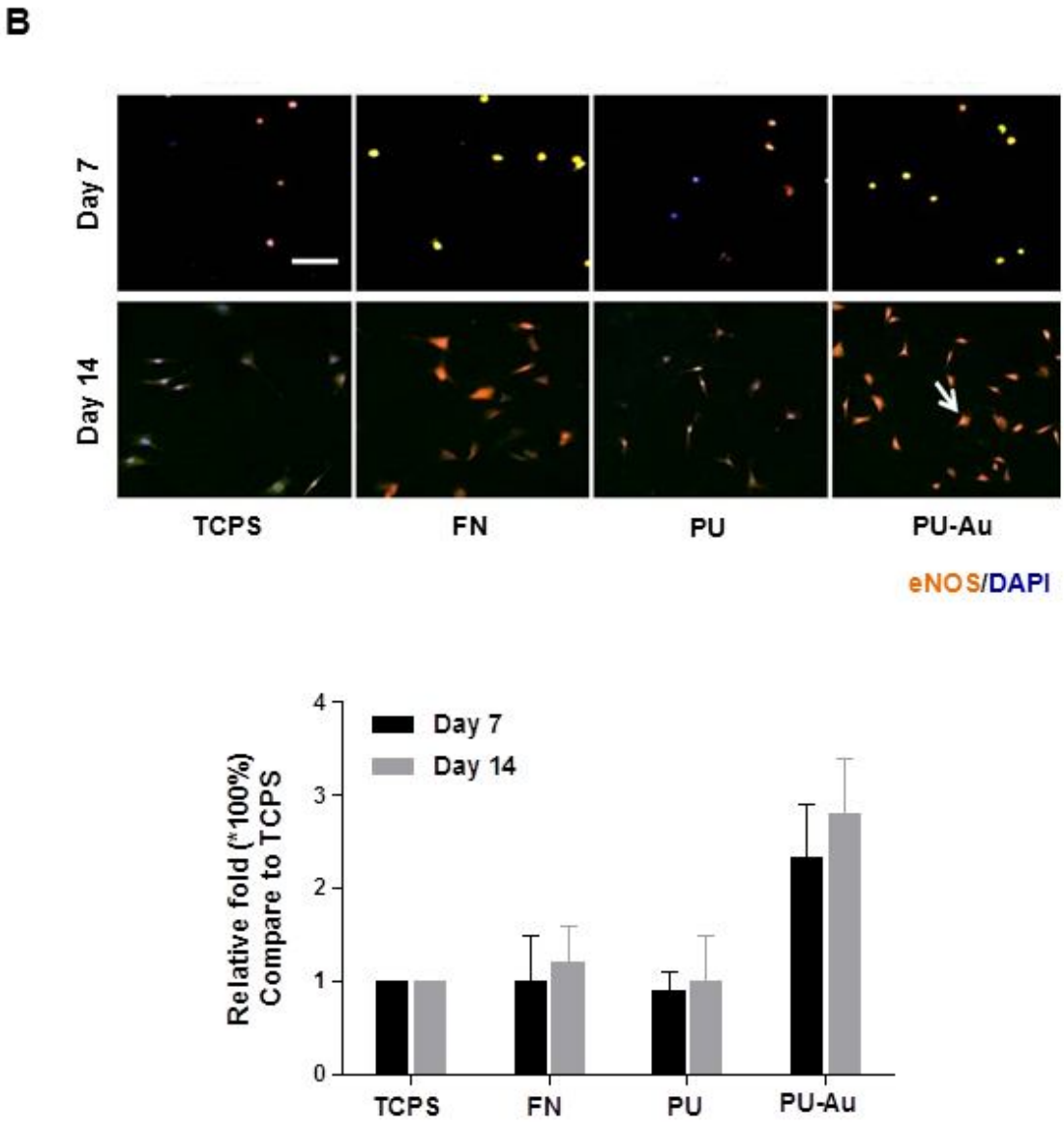
Fig. 3



<**Figure 3**>: **(A)** The expression of mRNA in ECs cultured on PU-Au nanocomposites for day1, day7, day14 and day21. RT-PCR experiment was represented. GAPDH was used as an internal control. Semi-quantitative measurement of RT-PCR for EPCs cultured on each material was showed. Mean ±SD. **(B)** The localization and distribution of eNOS protein in EPCs visualized by fluorescent microscopy. Cells were cultured on materials for day 7 and day 14 and were fixed for immunofluorescence analysis. Cells were first incubated with anti-eNOS antibody followed by incubation with the secondary cyc5.5-conjugated immunoglobulin (red color fluorescence). The cell nuclear staining was performed by DAPI (blue color staining). Scale bar: 50 μm. Arrows indicate where the eNOS protein expressed.

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Fig. 4

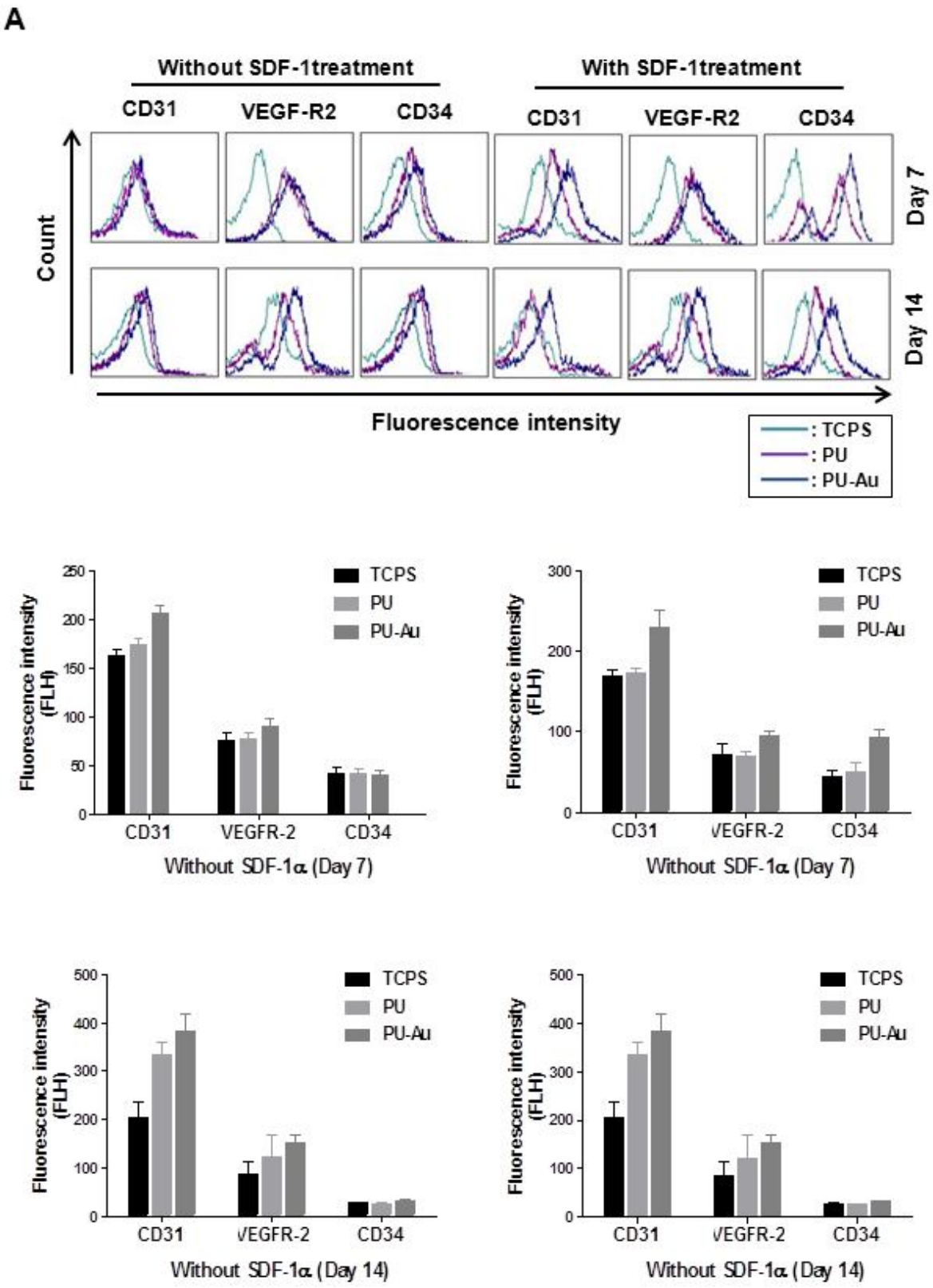

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Fig. 4

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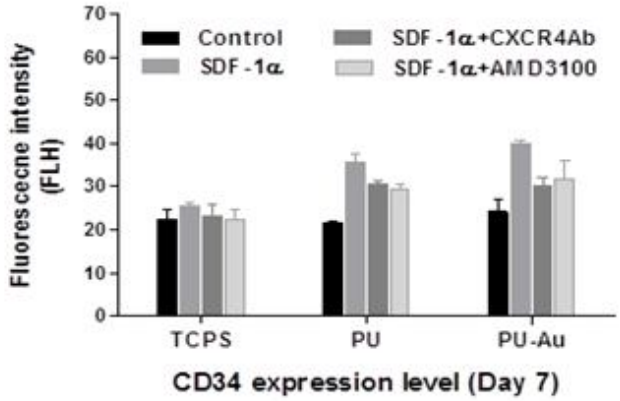

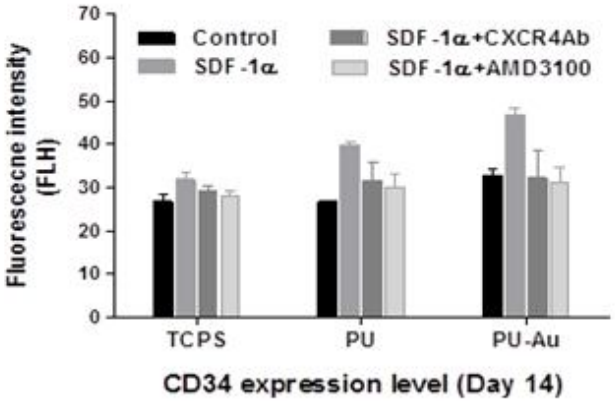

<**Figure 4**>: **(A)** FACS analysis of CD31, CD34 and VEGF –R2 of EPCs with or without VEGF (50 ng/ml) and SDF-1 (50 ng/ml) treatment. EPCs were seeded on materials, and cultured with VEGF and SDF-1 for day 7 and day14. Semi-quantitative measurement of RT-PCR for EPCs cultured on each material was showed. Mean ±SD. **(B)** Cells were pretreated with CXCR4-Antibody and AMD3100 for 1 h. Semi-quantitative measurement by flow cytometry shows significantly reduced CD31, CD34 and VEGF-R2 surface marker expressions for EPCs on PU-Au after CXCR-antibody and AMD3100 pre-treatment. Data are mean ± SD. * $p < 0.05$: greater than TCPS; ** $p<0.05$: greater than PU.

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Fig. 5

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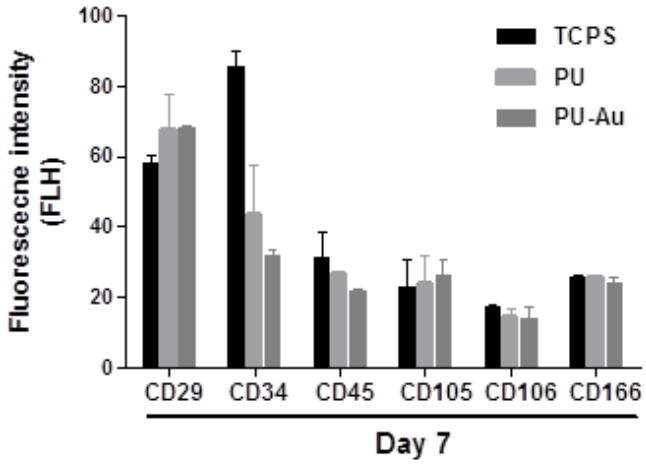

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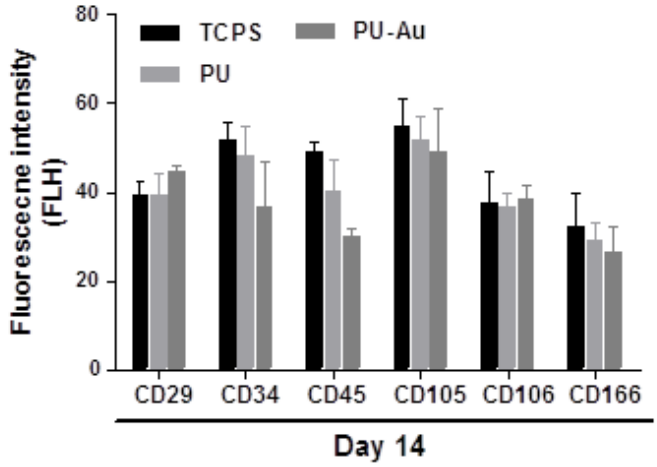

<**Figure 5**>: The expression of cell surface markers ( ) was examined by FACS analysis.

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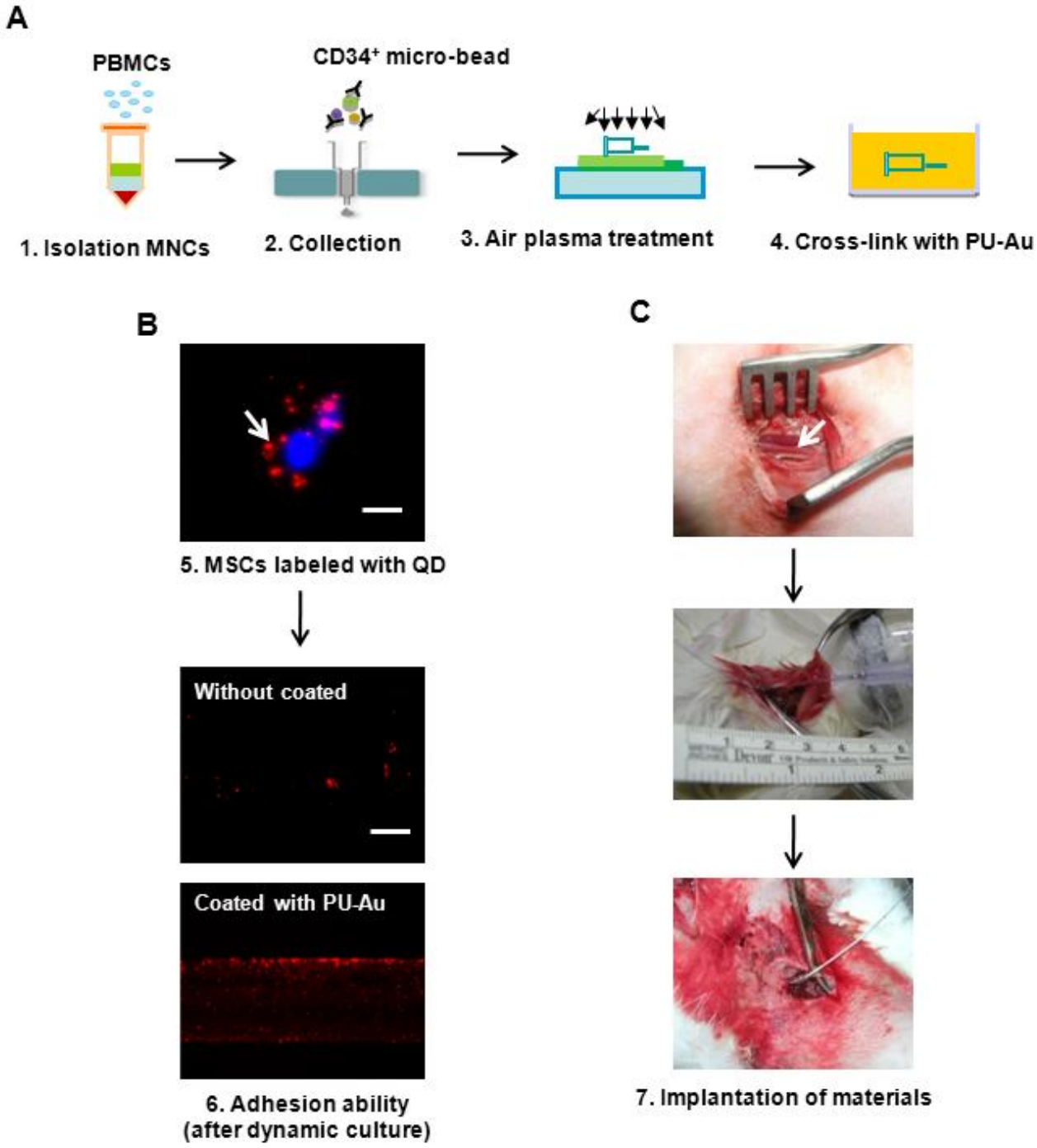
Fig. 6



<**Figure 6**>: **(A)** Surface grafting of PU-Au. The surface catheter coated with PU-Au nanocomposites was grafted by plasma equipment. **(B)** Cells seeding and maintenance of PU-Au in vitro. Cells were labeled with fluorescence tracer dye (Quantum dot, QD) at a concentration of 10 nM. After dynamic culture for 48 hrs, materials were checked for their attachment ability under fluorescence microscope before implantation materials into in vivo. Scale bar = 20 μm. **(C)** Implantation of cell-seeded materials into rabbit femoral artery.

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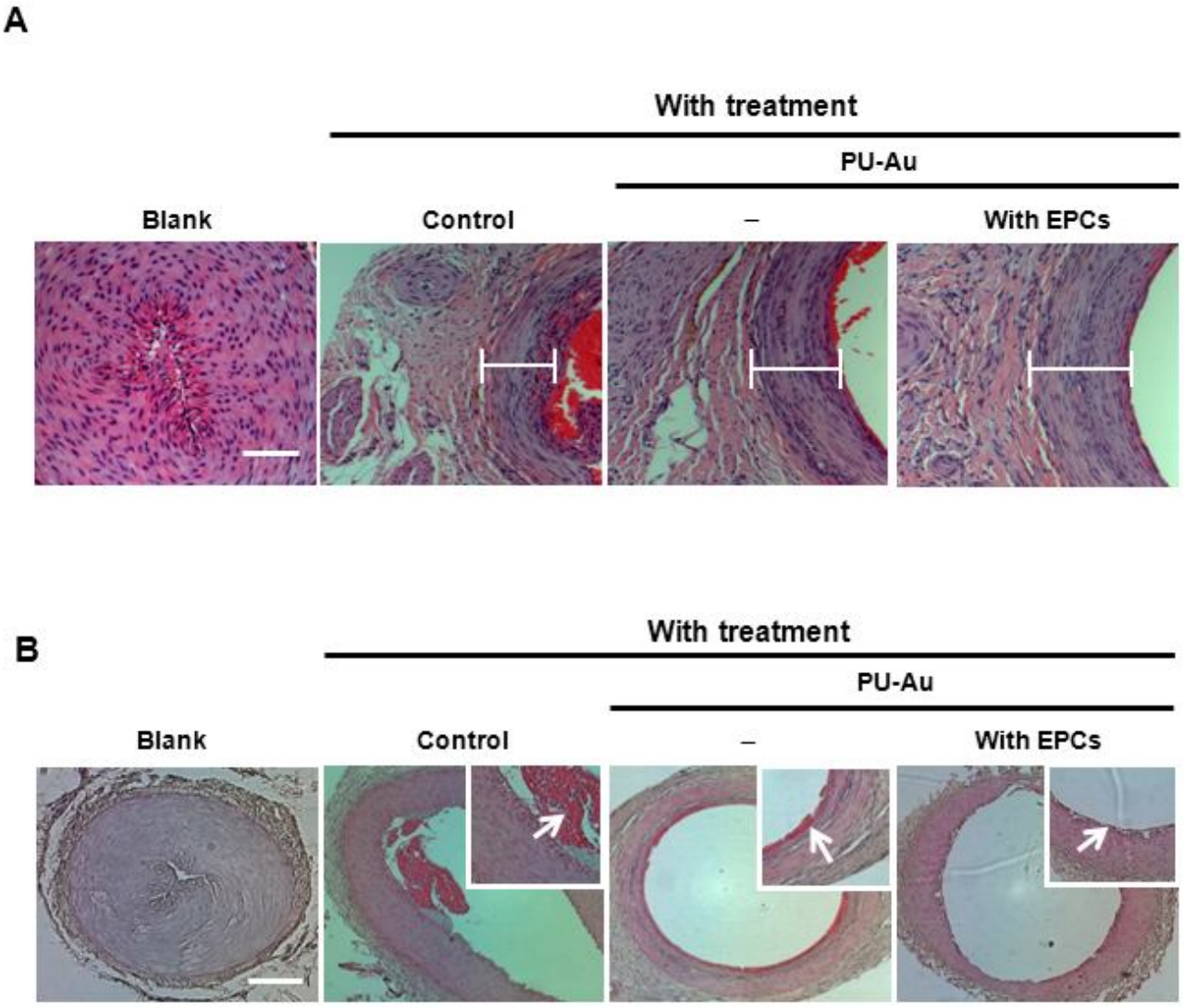
Fig. 7



<**Figure 7**>: Cell organization and anti-thrombogenic property after 28 d for EPCs implantation on PU-Au nanocomposites vascular graft in vivo. The samples were stained with Hematoxylin/eosin (H&E) staining staining is shown. Images are representative from at least four independent groups. (**A**). Vascular tissue morphology and (**B**). anti-thrombogenic property of cross-section in a cellular graft **(a)** Blank (without treatment), **(b)** Control (with treatment), **(c)** PU-Au (with treatment) and **(d)** PU-Au + EPCs (with treatment). Scar bar = 50 μm.

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Fig. 8

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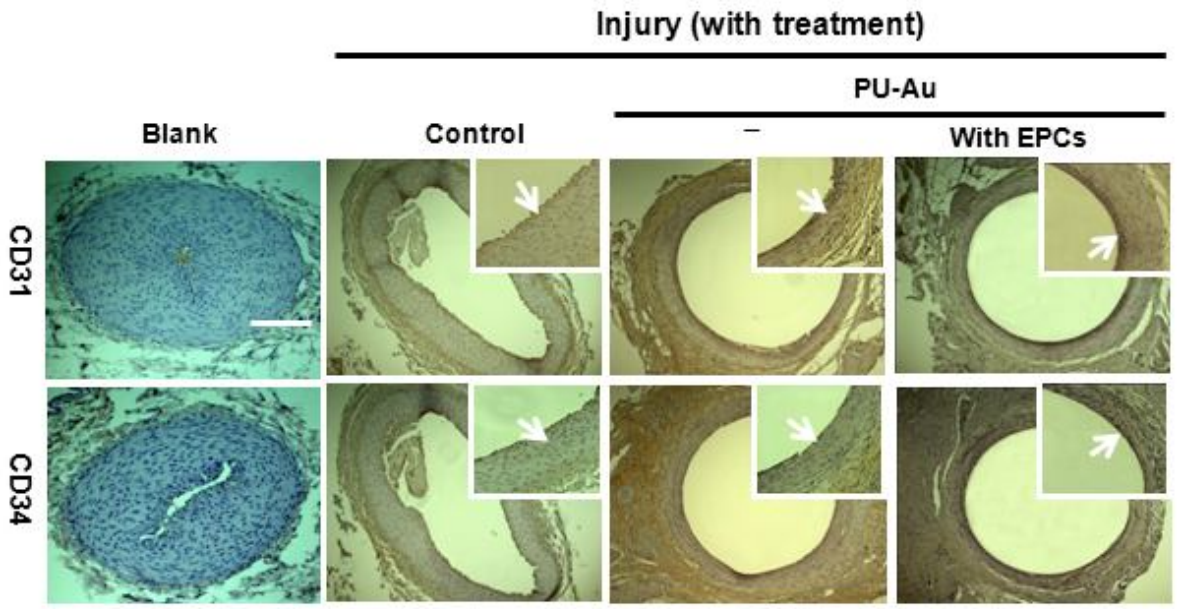

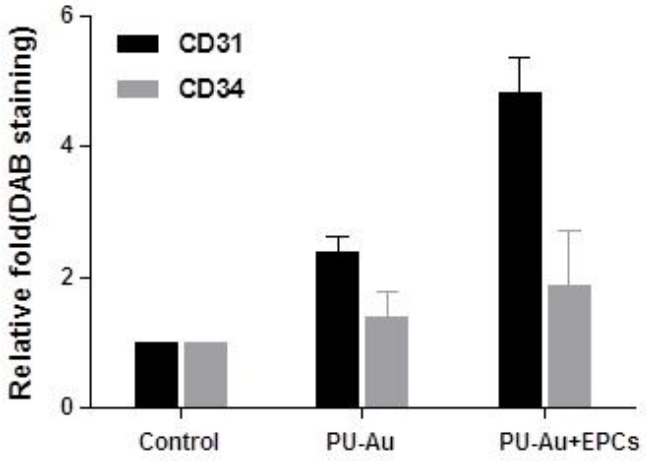

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Fig. 8

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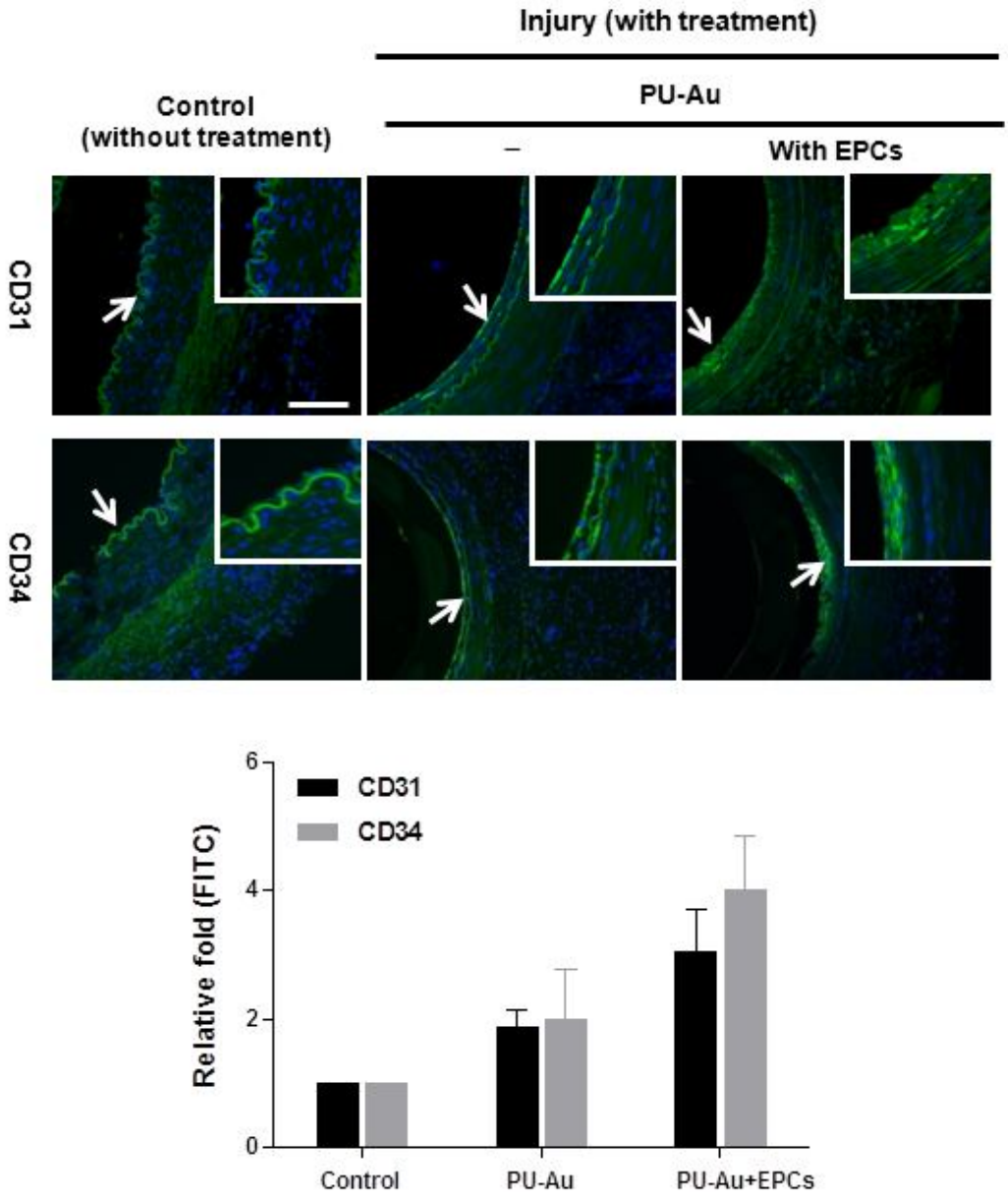

<**Figure 8**>: PU-Au nanocomposites promoted ECPs to differentiate into ECs after repairing rabbit femoral artery injured by wire. EPCs were incubated with PU-Au or not for 48 hr and implantation into injured artery. The injured artery were separated from rabbit and immunostainined with anti-CD31 antibody and CD34 antibody, cells followed by stained with **(A)** 3,3-Diaminobenzidine (DAB) and stained with **(B)** FITC-conjugated immunoglobulin (green color fluorescence), cell nuclear staining was performed by 4', 6'-diamidino-2-phenylindole (DAPI) (blue color staining) (indicated by thin arrows). Bar = 50 μm.

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國科會補助計畫衍生研發成果推廣資料表

日期:2011/10/25

國科會補助計畫	計畫名稱: 探討血管與缺血性腦中風疾病:不同新穎複合生醫奈米材料對血管新生之分子機轉 (SDF-1/CXCR4)及治療策略發展研究
	計畫主持人: 洪慧珊
	計畫編號: 98-2314-B-039-029- 學門領域: 幹細胞/再生生物醫學

無研發成果推廣資料

98 年度專題研究計畫研究成果彙整表

計畫主持人：洪慧珊	計畫編號：98-2314-B-039-029-
計畫名稱：探討血管與缺血性腦中風疾病:不同新穎複合生醫奈米材料對血管新生之分子機轉(SDF-1/CXCR4)及治療策略發展研究	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		

其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)	研究成果: 研討會論文: 1. 2009 年第二屆泛太平洋國際幹細胞研討會 (The 2nd Pan Pacific Symposium on Stem Cells Research 2009) Hung, HS &amp； Hsu, SH Nanogold composites promote the maturation driven by endothelial progenitor cells，壁報論文 (Recipient of First prize in Best Poster Award)。 2. 2010 年台灣幹細胞學會第六屆年會 (Taiwan Society for Stem Cell Research (2010) 邀請專題演講: Hung, HS, Polyurethane-Gold Nanocomposites Promote the Maturation of Endothelial Progenitor Cells Partially through Stromal Derived Factor- α (SDF-1α)/CXCR4 Signaling Pathway in the Vascular Disease Model。 學術發表： 1. Huey-Shan Hung； Mei-Yun Chu； Chien-Hsun Lin； Chia-Ching Wu； Shan-Hui Hsu Mediation of the migration of endothelial cells and fibroblasts on polyurethane nanocomposites by the activation of integrin-focal adhesion kinase signaling. Journal of biomedical materials Research (JBMR A), 2011, In press. 2. Huey-Shan Hung, Yi-Chun Yang, Mei-Yun Chu, Chien-Hsun, Lin, Yu-Jun Lin, Shan-hui Hsu. Polyurethane-gold nanocompsoites promote the maturation of endothelial progenitor cells partially through stromal-derived factor-1)/CXC4 signaling pathway in the vascular disease model (In preparation).

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項目	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	
	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：□已發表 □未發表之文稿 ■撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

先前研究發現具有奈米特徵之聚胺酯（polyurethane； PU）基材上之可以有效促進內細胞之移動（migration）速率及增殖（prioliferation）效應，對於受損血管組織之治療方向與策略也更進一步提供具有實用性與應用性之明確方向。此計畫中我們將內皮前趨細胞（endothelial progenitor cells, EPCs)，培養於奈米基材上探討其影響分化能力並促進成為成熟內皮細胞之相關訊息調控機轉，期能解決內細胞的之來源不足的問題，提供體內實際內皮化之速度之體內動物實驗研究模式。

一、 成效評估

綜合上述之研究成果發現，本計畫在學術發表上，PU-Au nanocomposites 已經發表了共 1 篇 (JBMR A, 2011, In press)，目前已有一篇論文已經撰寫趨於完成 (Title: Polyurethane-gold nanocompsoites promote the maturation of endothelial progenitor cells partially through stromal-derived factor-1 脉)/CXC4 signaling pathway in the vascular disease model)，正在準備投稿。

二、檢討與展望

由於在製成生醫奈米材料應用於組織過程中，血液幹細胞的來源取得及其分化增殖效應仍面臨所多限制，在外來都需要更進一步修正與開發其他合適可以替代的幹細胞來源，並且尋找除了 polyurethane 外是否有其他更好生物相容性的高分子可以做為仍工血管表面

改質好的生醫材料。