A suspension of porcine cartilage extra-cellular matrix exerts anti-angiogenic effect in a rabbit corneal model

Hee-Woong Yun[1,2], Sun Young Kim[1,2], Bo Ram Song[1,2], Tae-im Kim[3], Byung Hyune Choi[4], Young Jick Kim[1], So Ra Park[5], Sang-Hyug Park[6], Byoung-Hyun Min[1,2,7]
[1]Cell Therapy Center, Ajou University School of Medicine, Suwon, Korea, [2]Department of Molecular Science and Technology, Ajou University, Suwon, Korea, [3]Department of Ophthalmology, Institute of Vision Research, Yonsei University College of Medicine, Seoul, Korea, [4]Department of Biomedical Sciences, Inha University College of Medicine, Incheon, Korea, [5]Department of Physiology, College of Medicine, Inha University, Incheon, Korea, [6]Department of Biomedical Engineering, Jungwon University, Goesan, Korea, [7]Department of Orthopedic Surgery, School of Medicine, Ajou University, Suwon, Korea

Statement of Purpose: Previously, we have shown that extra-cellular matrix (ECM) secreted from articular chondrocytes has an inhibitory effect on endothelial cells adhesion and vessel invasion *in vitro* and *in vivo*. In this study, we fabricated a suspension form of porcine-cartilage-derived ECM powder (PCP-s) and investigated its anti-angiogenic activity *in vitro* and its *in vivo* effect on the corneal angiogenesis in a rabbit model.

Methods: PCP was prepared from the porcine cartilage via multiple processes, including pulverization, decellularization, pepsinization, lyophilization and sterilization. Finally, PCP was dissolved in saline to prepare the suspension (PCP-s) of up to 10mg/ml. Tube formation assay was performed using human umbilical vein endothelial cells (HUVECs) to examine its anti-angiogenic activity *in vitro*. Corneal angiogenesis was induced in 20 rabbits by suturing (8-0 silk suture) at 3mm distance from the limbus. Rabbits were then randomly divided into 4 groups based on treatment materials: group 1, saline; group 2, 10 mg/ml of type I collagen suspension; group 3, 10 mg/ml of PCP-s and group 4, 15 mg/ml bevacizumab. The treatments were administered (topical application, 50ul) three times a day for 7 consecutive days. On day 7, digital photographs of the cornea were taken by camscope (Somethech Vision, Seoul, Korea). The corneal vessel invasion was assessed using an image pro software. For histological analysis, cornea tissues sections were prepared and stained with H&E. Four sequential sections were used to count the number of vascular channels per 1 micro-meter square in each group. Immunohistochemical analysis was performed to evaluate the expression of vascular endothelial growth factor (VEGF) and CD-31 as angiogenic markers.

Results: In the tube formation assay *in vitro*, PCP-s inhibited efficiently vessel formation of HUVECs *in vitro* (Figure 1). In the corneal angiogenesis model *in vivo*, the group treated with PCP-s showed decreased vessel invasion area compared to the saline and type I collagen groups (Figure 2 (A); $p<0.001$). The H&E staining of tissue sections indicated that the PCP-s group had reduced vessel number compared to the saline and type I collagen groups (Figure 2 (B); $p<0.01$ and $p<0.05$, respectively). The expression of VEGF and CD-31 in the subepithelial stroma was also less in the PCP-s group than in the other groups in the immunohistochemical analysis (Figure 3).

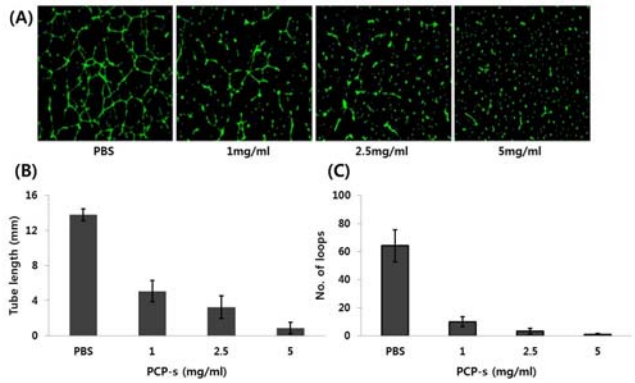

Figure 1. (A) Calcein AM staining images, (B) The length (C) and the number of tubes

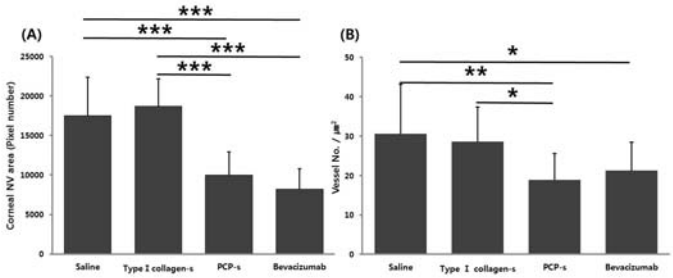

Figure 2. (A) Corneal neovascular (NV) area, (B) Number of vascular channel

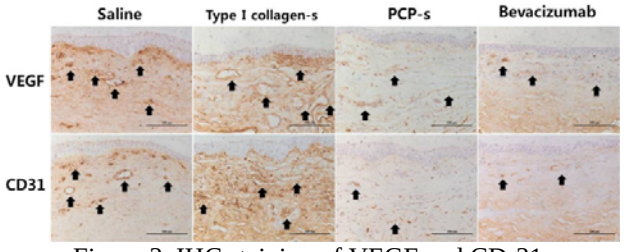

Figure 3. IHC staining of VEGF and CD-31

Conclusions: In this study, PCP-s showed anti-angiogenic effect on the HUVECs *in vitro*. In addition, PCP-s reduced corneal angiogenesis according to microscopical and histological evaluations. We speculate that PCP-s could be a useful therapeutic tool for anti-angiogenic applications including the prevention of corneal angiogenesis.

References: 1. Kyoung-Hwan Choi. Tissue Eng Regen Med. 2012;9(1):43-50. 2. Byung Hyune Choi. Biomaterials. 2014;35: 5711e5720. 3. Patra, D. and L.J. Sandell. Expert Rev Mol Med. 2012;14:e10.