Cellular responses of smooth muscle cells to epigallocatechin gallate-releasing bioresorbable polymer and its cellular mechanism

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Introduction: A major complication of coronary stenting is restenosis, often accompanied by inflammatory reactions and smooth muscle cell proliferation and migration. (−)-epigallocatechin-3-*O*-gallate (EGCG), a major polyphenolic constituent of green tea, has been shown to exert anti-thrombotic, anti-inflammatory and anti-proliferative activities [1,2]. In this study, it was hypothesized that the sustained release of EGCG from bioresorbable poly (lactic acid-*co*-ε-caprolactone, PLCL) would reduce the proliferation and migration of vascular smooth muscle cells (VSMCs), and nuclear factor-κB (NF-κB) might be involved as a mechanism of this reduction by EGCG.

Materials & Methods:

Preparation of EGCG-releasing PLCL (E-PLCL) copolymer films: Bioresorbable, non-porous PLCL (75:25=mol/mol, MW 130,000 ~ 160,000) thin films (9 mm in diameter and 0.1 mm in thickness) used in this study were kindly supplied from BMG Inc. (Kyoto, Japan). E-PLCL copolymers were fabricated by mixing PLCL with 5 and 10 wt% EGCG (TEAVIGO™, DSM Nutritional Products) in acetone at 60ºC.

Primary culture of vascular smooth muscle cells from rat aorta: VSMCs were obtained by limited enzymatic digestion from the tunica media of rat thoracic aorta as previously reported (3).

EGCG release from E-PLCL copolymer: E-PLCL films were attached to the bottom of a glass vial by using vacuum grease and then incubated in phosphate-buffered saline (PBS, pH 7.4) at 37°C for 15 days. At the end of each pre-determined incubation period, the concentration of EGCG released from the copolymer in media was quantified by absorbance at 275 nm in a UV spectrophotometer.

Cell attachment and proliferation assays: VSMCs were seeded onto intact PLCL and E-PLCL films at a seeding density of 1×10^4cells/cm^2. Cell attachment at 6h and cell proliferation at 1 and 3 days were determined by WST method. VSMC attachment and proliferation were found to be directly proportional to the metabolic reaction products obtained in water soluble tetrazolium salt (WST-8, Dojindo Lab., Kumamoto, Japan). The absorbance was determined at 450 nm in a microplate reader. In addition, proliferated cell morphologies were observed after 3days by a immunocytochemical analysis.

Cell migration assay: VSMCs were seeded onto 48-well plates at density of 2×10^4 cells/well and then grown to confluence. Monolayers were scraped (denuded) using a 1 ml plastic micropipette tip, and replaced with or without conditioned media obtained by incubating E-PLCL in the fresh media. The cells were incubated and
then visualized for the cells that migrated toward denuded space for 72 h by immunocytochemical analysis.

Results & Discussion:

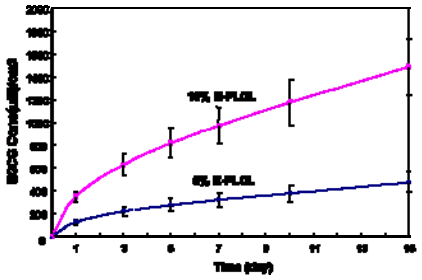

Figure 1. In vitro release of EGCG from E-PLCL copolymer

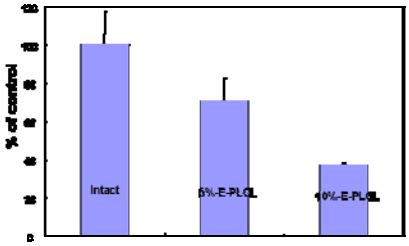

Figure2. Proliferation of VSMCs onto intact and E-PLCL films at 3 days

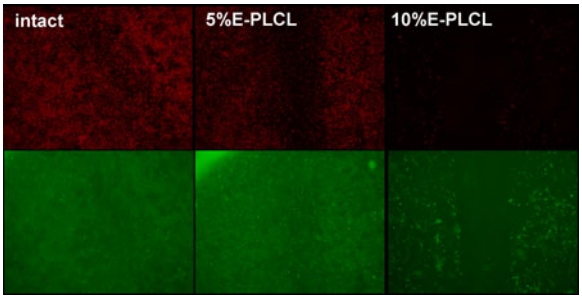

Figure 3. Migration of VSMCs onto intact and E-PLCL films at 3 days after cell scratch.

As shown in figure 1, EGCG was sustainedly released from E-PLCL. The proliferation of VSMCs onto E-PLCL was significantly inhibited in spite of serum stimulation (figure2). Recovery of denuded area by VSMCs receiving conditioned media obtained from 10%E-PLCL was significantly inhibited after 72 h, whereas VSMCs without conditioned media migrated into denuded area showing complete recovery onto intact PLCL (figure 3). In VSMCs cultured onto E-PLCL, furthermore, the expression of NF-κB completely disappeared. These results suggest that the inhibitory effect of EGCG released from bioresorbable polymers on VSMC behaviors may be mediated through NF-κB suppression, and these EGCG-releasing polymers can be applied for fabricating an EGCG-eluting vascular stent.

References:

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2. Maeda K et al. Atherosclerosis 2003;166(1):23-30
3. Hwang KC et al. J. Cardiovasc. Pharmacol. 2002;39: 271-277.