

## Angiogenesis evaluated by monochromatic synchrotron radiation microangiography

Hidezo MORI<sup>1,2</sup>, Etsuro TANAKA<sup>\*1</sup>, Naoichiro HATTAN<sup>1</sup>, Yoshiro SHINOZAKI<sup>1</sup>, Naoto FUKUYAMA<sup>1</sup>, Hideki IWAGURO<sup>1</sup>, Yoshinori SUGIO<sup>1</sup>, Takafumi SEKKA<sup>1</sup>, Masanori ISHII<sup>1</sup>, Hisanori FUJIKURA<sup>1</sup>, Eriko SATO<sup>1</sup>, Hirofumi KASAHARA<sup>1</sup>, Koji KUMURA<sup>1</sup>, Eriko KUWABARA<sup>1</sup>, Yoshiro IWATA<sup>1</sup>, Munetake KANDA<sup>1,2</sup>, Kenkichi TANIOKA<sup>3</sup>, Norihumi EGAMI<sup>3</sup>, Misao KUBOTA<sup>3</sup>, Kazunori MIYAKAWA<sup>3</sup>, Yuji OHKAWA<sup>3</sup>, Nobuo SAITO<sup>3</sup>, Hiroshi OHTAKE<sup>3</sup>, Ryo MOCHIZUKI<sup>4</sup>, Kouichi YAMAGUCHI<sup>4</sup>, Toshiaki KAWAI<sup>5</sup>, Katsuhiko SUZUKI<sup>5</sup>, Kinji TAKASE<sup>5</sup>, Hiroki KAWAKAMI<sup>5</sup>, Kazuyuki HYODO<sup>6</sup>, Masami ANDO<sup>6</sup>

<sup>1</sup>Tokai University School of Medicine, Bohseidai, Isehara-shi, Kanagawa 259-1193, Japan

<sup>2</sup>National Cardiovascular Center Research Institute, Fujishirodai, Suita-shi, Osaka 565-8565, Japan

<sup>3</sup>NHK Science & Technical Research Laboratories, Kinuta, Setagaya-ku, Tokyo 157-8510, Japan

<sup>4</sup>NHK Engineering Services, Inc., Udagawa, Shibuya-ku, Tokyo 150-0042, Japan

<sup>5</sup>Hamamatsu Photonics K.K., Toyooka, Iwata-gun, Shizuoka 438-0193, Japan

<sup>6</sup>Institute of Material Structure Sciences, National Laboratory for High Energy Physics, Oho, Tsukuba-shi, Ibaraki 305-0801, Japan

We examined the effects of biodegradable gelatin hydrogel (GHG) on angiogenesis following hindlimb ischemia in 17 rabbits by monochromatic synchrotron radiation microangiography. At 10 days after femoral arterial removal either of 3 gene therapies was performed; GHG-FGF4 (fibroblast growth factor 4), naked FGF4, and GHG-lacZ (Control). Four weeks after the gene transfer, lower limb necrosis and muscle atrophy were ameliorated in two FGF4-treated groups than in GHG-lacZ group ( $P < 0.05$  Kruskal-Wallis test).

Collateral vessel development was evaluated with a Synchrotron radiation excited microangiography as previously described (Mori et al, 1996). Briefly, monochromatic synchrotron radiation with an energy level of 33.3 keV was used as the X-ray source, and contrast images of the object were formed on a high-sensitivity fluorescent screen (FOS, Hamamatsu Photonics Co., Hamamatsu, Japan), which was scanned at 30 frames/sec by a high-definition TV camera with 1125 TV lines (New Super HARP, NHK, Japan). This system is capable of separating adjacent lead lines only 20  $\mu\text{m}$  apart on the resolution bar chart. The contrast material containing 37% non-ionic iodine (iopamidol, Nihon

Schering Co., Tokyo) was injected (1 ml/s for 5 s) via a 4F catheter as above under administration of adenosine (100 $\mu\text{g/kg/min}$ ) or acetylcholine (20 $\mu\text{g/kg/min}$ ) via the same catheter. The vessel density in the ischemic area was evaluated as an angiographic score.

Synchrotron radiation microangiography revealed that administration of adenosine induced vasodilation of microvessels in GHG-FGF4-treated rabbits. In GHG-lacZ-treated rabbits, the vascular density rather decreased by adenosine treatment. The vascular density ratio (adenosine / baseline) evaluated by synchrotron radiation was the highest for GHG-FGF4 group ( $1.33 \pm 0.11$ ) and decreased to  $1.06 \pm 0.15$  and to  $0.86 \pm 0.1$  in naked FGF4 group and GHG-lacZ group.

These observations indicate a difference in maturity of angiogenic vascular networks between the three groups.

Acknowledgments: This work was partially supported by NEDO.

### References

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\* tanaka@is.icc.u-tokai.ac.jp