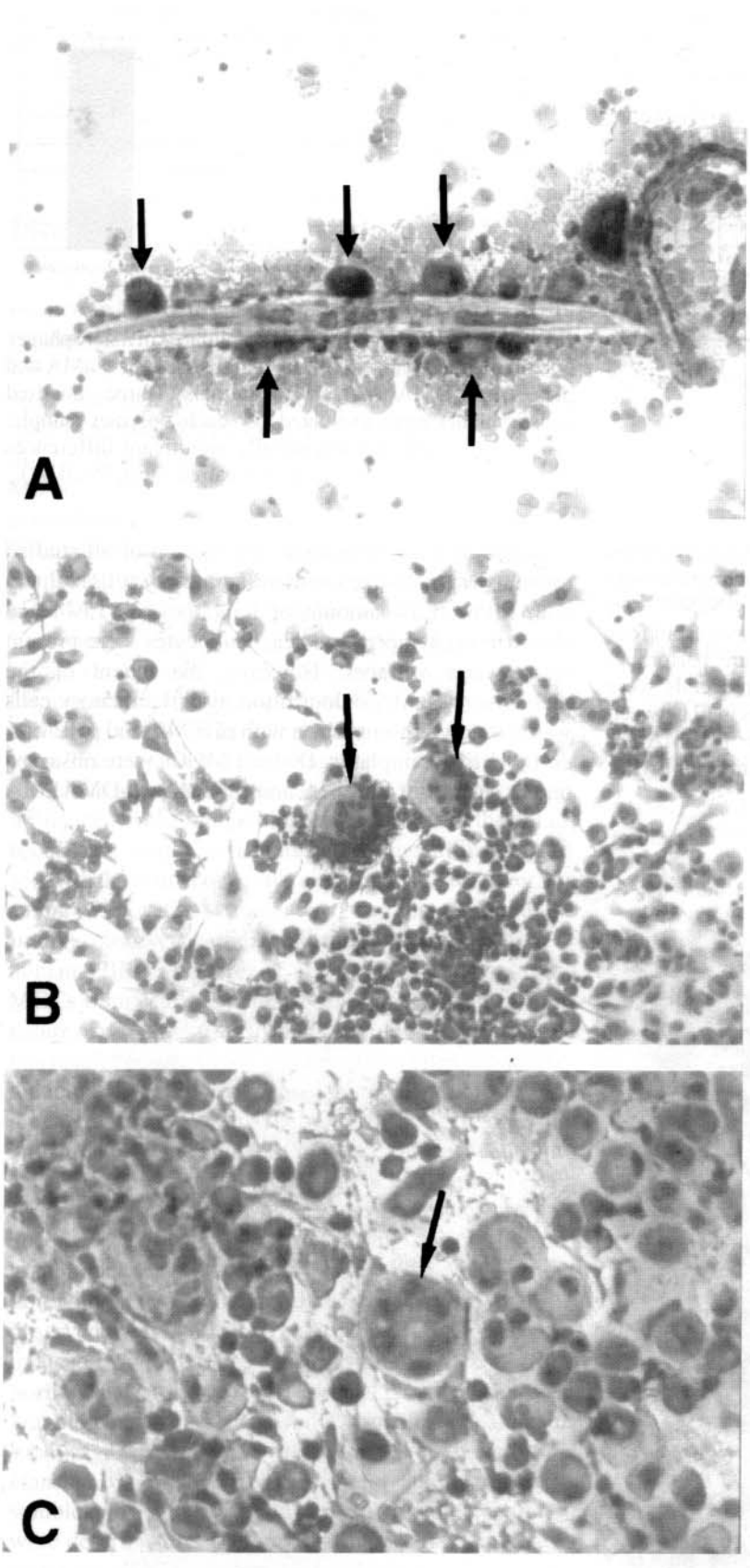

Fig. 2. A – MGC (arrows) formation around the nematode. B, C – foreign-body MGCs of Langhans type formed in the dish without direct contact with pHEMA-*co*-DMAEMA beads *in vitro*. Staining according to the Pappenheim procedure, magnification 100× (A), 200× (B), 400× (C).

the specimens were washed with phosphate-buffered saline, fixed with methanol, stained by the Pappenheim method and evaluated under a light microscope. The experiments were performed separately with cells collected from four healthy donors each in duplicate. The PBMCs cultured without the *NB* or polymer beads were employed as a control. The fusion index (FI) (Smetana et al., 1990; Seitzer et al., 1997) was calculated according to the equation:

FI = number of nuclei in MGC / total number of nuclei (1)

Implantation experiment

The strips prepared from each material were subcutaneously implanted into the interscapular region of male Wistar rats (Velaz, Prague, Czech Republic) under aether anaesthesia and sterile conditions (Smetana, 1987). For each material, six rats were employed. The strips were harvested 8 days after the surgery, when the extent of foreign-body reaction in the rat is very extensive (Smetana, 1987). The cells on the strip surfaces were fixed with paraformaldehyde and stained with haematoxylin. The fusion potential of MPhs on the implant surface was estimated by calculating the fusion index (FI).

Results

In vitro, all the types of tested beads (pHEMA, pHEMA-*co*-DMAEMA, pHEMA-*co*-NaMA) were surrounded by cells and the cells even colonized the bead surfaces. The highest number of cells was accumulated around the beads prepared from pHEMA-*co*-DMAEMA (Fig. 1, BD-30). The MGCs were observed beginning day 4 of the cultivation. Some MGCs colonized even the surface of the tissue culture dish without contact with the bead surface (Fig. 2). The level of FI was not significantly influenced by the bead composition (Fig. 3). The level of the fusion index (0.50 ± 0.11) on day 8 of the control experiment performed with *NB* clearly demonstrated the extensive formation of MGCs in granuloma-like cell accumulations induced by the nematode and it was significantly higher ($P < 0.01$) than FI in the polymer experiment.

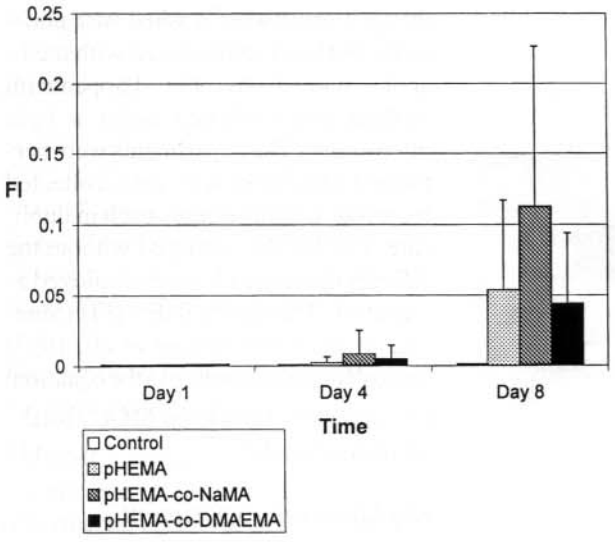

Fig. 3. Level of fusion index (FI, mean ± SD) calculated for PMBCs cultured without polymer samples (control) and with beads prepared from pHEMA, pHEMA-*co*-NaMA and pHEMA-*co*-DMAEMA. PMBCs were collected from four healthy volunteers and the experiments were performed separately, each in duplicate. Three hundred cells were evaluated in each experiment. The differences in FI between the pHEMA and pHEMA-*co*-NaMA or pHEMA-*co*-DMAEMA were statistically insignificant employing Student's paired t-test.

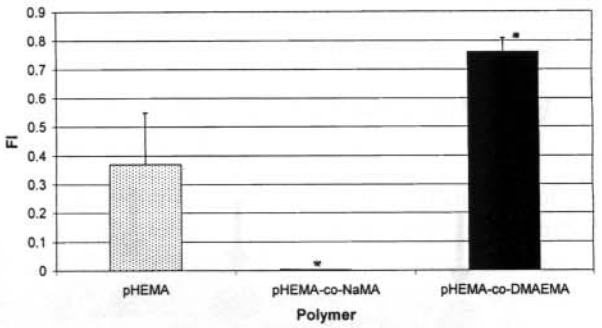

Fig. 5. Level of fusion index (FI) calculated in macrophages colonizing the surface of pHEMA, pHEMA-*co*-NaMA and pHEMA-*co*-DMAEMA. Six animals (three hundred cells/implant) were evaluated for each polymer sample. Asterisks indicate the statistically significant differences from the pHEMA level (Student's paired t-test, $P < 0.01$).

In the *in vivo* experiment, the surface of all studied implants was colonized with inflammatory cells, namely with MPhs. Low amount of lymphocytes (15%) and almost no polymorphonuclear leukocytes were present on polymer surfaces. However, the extent of the pHEMA-*co*-NaMA colonization by inflammatory cells was very low in comparison with pHEMA and pHEMA-*co*-DMAEMA implants. Distinct MGCs were observed on the surface of pHEMA and pHEMA-*co*-DMAEMA

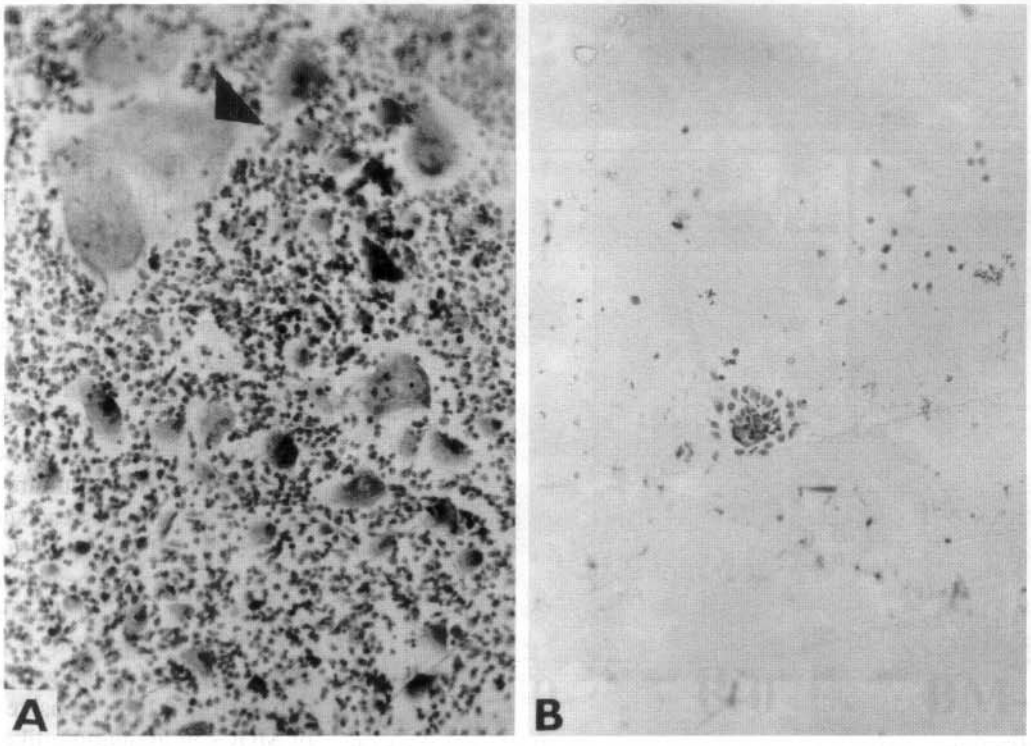

Fig. 4. Surface of pHEMA (A) and pHEMA-*co*-NaMA (B) implant 8 days after the surgery. A typical foreign-body MGC is designated by an arrowhead. The surface of implants is not stained. Haematoxylin staining, magnification 200×.