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Morphometrical analysis of multinucleated giant cells in subdermal implants of poly-lactic acid in rats

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Abstract The use of bioabsorbable polymers in (bio)medical applications has increased greatly in recent years, mainly because of their good bioreabsorption and biocompatibility. In this work, we examined the development of foreign body giant cells in intimate contact with porous membranes of poly L–lactic acid containing 7% of plasticizer triethylcitrate implanted in the backs of rats. The membranes were removed 2, 7, 14, 21, 28, 60, 90 and 180 days after implantation, along with a portion of the tissue around the implant. Histological analysis of the implant and tissue revealed the formation of a fibrous capsule from the seventh day of implantation onwards. Foreign body giant cells appeared from the seventh day and increased in number up to the twenty-eighth day and then up to the ninetieth day of implantation, remaining constant up to the end of the study onwards, and increased in number up to the ninetieth day after implantation and then remained constant. The number of nuclei in these cells increased from the seventh day of implantation up to the ninetieth day and then up to the end of the study.

Introduction

The relationship of organs or tissues to materials with a temporary structural function is a rapidly expanding field of research. In recent years, reabsorbable polymers have gained increasing importance in (bio)medical uses because of their good bioreabsorption and biocompatibility.

Poly L-(lactic acid) (PLLA) is a bioreabsorbable polymer synthesized from monomers that are part of natural metabolic processes of organisms [1, 2]. PLLA has several potential applications that vary according to the type of implants, and include spongy structures (DrylacTM) [3], pins [4], and double layer [5], monolayer [6] and mixed material [7] matrices.

The addition of the plasticizer triethylcitrate to PLLA alters the flexibility of the material and results in the formation of pores in the polymer. This porosity contributes to the control of polymer degradation and allows the adhesion and migration of connective tissue cells to the membrane pores, an important characteristic in the rebuilding of tissue [6, 8].

A tissue reaction to PLLA involves a variety of cells, including fibroblasts, lymphocytes, mastocytes, foreign body giant cells, eosinophils and lymphoid cells [9]. The immunological response to prosthetic biomaterials is characterized by a rich macrophage inflammatory infiltrate and the formation of multinucleated giant cells [10]. The inflammatory response modifies the activity of macrophages, which are fundamental in the tissue reaction [11, 12], and stimulates the production of factors involved in the synthesis of collagen by fibroblasts [13]. Macrophages can be activated by a variety of stimuli and assume different forms, they can fuse to form multinucleated giant cells [14, 15]. The fusion of macrophages is induced by cytokines such as interleukin 4 and gamma-interferon [12, 15], but little is known about the mechanism involved [16].

In this work, we examined the histological response to the implant of PLLA membranes containing plasticizer attempting to the formation and development of foreign body giant cells around the membranes. The number of nuclei in these cells was also determined.

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Materials and methods

Production of implants

PLLA (MW 300,000) was provided as pellets by Medsorb Technologies International L.P. (Cincinnati, OH, USA). Ten grams of polymer were dissolved in 100 mL of methylene chloride (CH_2Cl_2, Merck) containing 7% triethylcitrate (Aldrich) in a closed recipient at room temperature [3, 17]. The mixture was then poured onto glass plates (100 cm^2 each) which were air dried (air flow of 1 L/min) at room temperature. After 15 h, the membranes were removed from the plates and vacuum dried for 24 h. Disks 5 mm in diameter and 620 μm thick were cut and used in the studies described below.

Implantation

The membranes were immersed in 70% ethanol and then vacuum dried. Sixteen female Wistar rats 3 months old were used. The rats were housed at 22 ± 2 °C on a 12 h light/dark cycle with food and water *ad libitum*. Two membranes were implanted in the dorsal subcutaneous tissue of rats ($n = 16$) anesthetized with ketamine and xylazine-HCl (16.6 and 3.33 mg/kg, i.p., respectively) (Virbac, Brazil). The health and behavior of the rats were assessed daily until sample collection 2, 7, 14, 21, 28, 60, 90 and 180 days post-implantation.

Light microscopy

Fragments of skin were fixed in Bouin solution and embedded in paraffin. Sections 5 μm thick were stained with Masson's trichromic and toluidine blue. Membrane fragments that had adhered to adjacent tissue were fixed in 4% paraformaldehyde and embedded in glycol methacrylate. Sections 2 μm thick were observed and photographed with a Nikon Eclipse E800 photomicroscope.

Histological analysis

The image analyses and the morphometry of foreign body giant cells and their nuclei were done using the program Image-Pro® Lite, version 3.0 for Windows. Three hundred and thirty images from each time of implantation magnified 200X (corresponding to 166 μm^2 each) were used to count the cells and nuclei. The slide images were chosen at random to which rat the tissue belonged.

Statistical analysis

The numbers of multinucleated giant cells and their nuclei were expressed as the mean ± standard deviation. Statistical comparisons among the groups were done using the non-parametric Kruskal-Wallis test. A p value < 0.05 indicated significance.

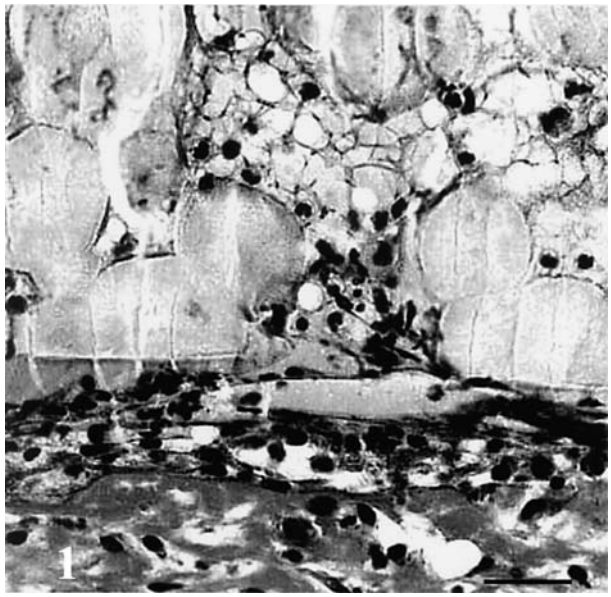

Fig. 1 Photomicrograph of PLLA membrane on second days post-implantation. Observe polymorphonuclear infiltrate, vascular edema and fibrin network. Bar: 50 μm.

Results

Microscopic analysis

In samples obtained two days after implantation, no fibrous capsule was observed around the polymer. A solid infiltration of polymorphonuclear cells surrounded by a fibrin network and edema was observed within the membrane pores (Fig. 1). No multinucleated giant cells were observed.

By seven days after implantation, a fibrous capsule containing fibroblasts and macrophages was observed on the surface of the implant. The invasion of tissue elements through the membrane pores and the presence of multinucleated giant cells were also observed (Fig. 2). An average of 1.11 ± 1.41 foreign body giant cells was observed per microscopic field, with 6.60 ± 4.64 nuclei per cell (Table 1).

Fourteen days after implantation, the histological analysis indicated invasion of tissue elements and foreign body giant cells by the membrane pores. An average of 1.67 ± 1.40 foreign body giant cells was seen per microscopic field, with 5.82 + 4.84 nuclei per cell (Table 1).

By 21 days, the capsule of connective tissue around the implants was well formed, with blood vessels close to the implant and penetrating the pores to enter the membrane. Tissue invasion occurred through the spaces among the

Table 1 Number of multinucleated giant cells and their nuclei as means ± standard deviation.

Days after implantation	Cells per field	Nuclei per cell
2	0.00	—
7	1.11 ± 1.41	6.60 ± 4.64
14	1.67 ± 1.40	5.82 ± 4.84
21	1.97 ± 1.26	6.75 ± 4.41
28	2.33 ± 1.23	6.52 ± 4.85
60	1.55 ± 1.16	8.21 ± 7.14
90	4.65 ± 3.60	9.60 ± 9.90
180	4.66 ± 3.23	13.48 ± 16.80

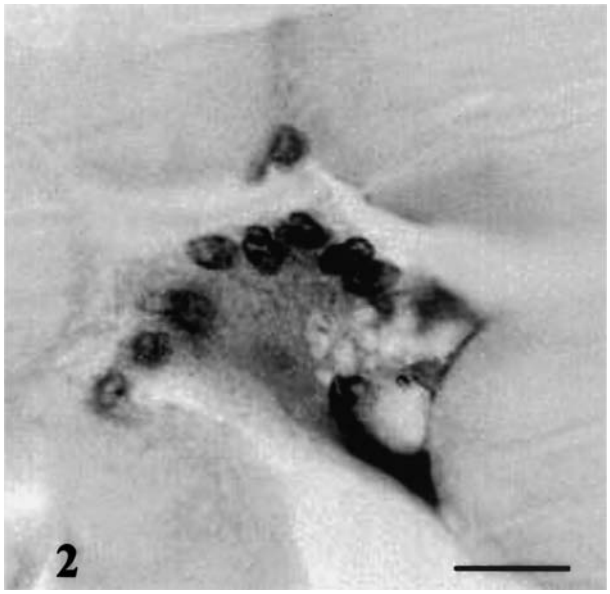

Fig. 2 Photomicrograph of PLLA membrane 7 days after implantation showing a multinucleated giant cell in contact with polymer fragments. Bar: 25 μm.

membrane units, in accordance with the distribution of these units along the implant surface. The extent of cellular invasion thus varied throughout the implant. Multinucleated giant cells were also present (Fig. 3). An average of 1.97 ± 1.26 foreign body giant cells was seen per microscopic field, with 6.75 ± 4.41 nuclei per cell (Table 1).

Twenty-eighth days after implantation, the analysis of the capsule revealed polymeric particles of different diameters involved in formation of the capsule, along with the presence of numerous blood vessels. Nerves were also observed within the polymer. An average of 2.33 ± 1.23 giant cells was seen per microscopic field, with 6.52 ± 4.85 nuclei per cell (Table 1).

After 60 days, intense degradation of the polymer resulted in globular fragments of various dimensions. An average of 1.55 ± 1.16 foreign body giant cells (Fig. 4) was seen per microscopic field, with 8.21 ± 7.14 nuclei per cell (Table 1).

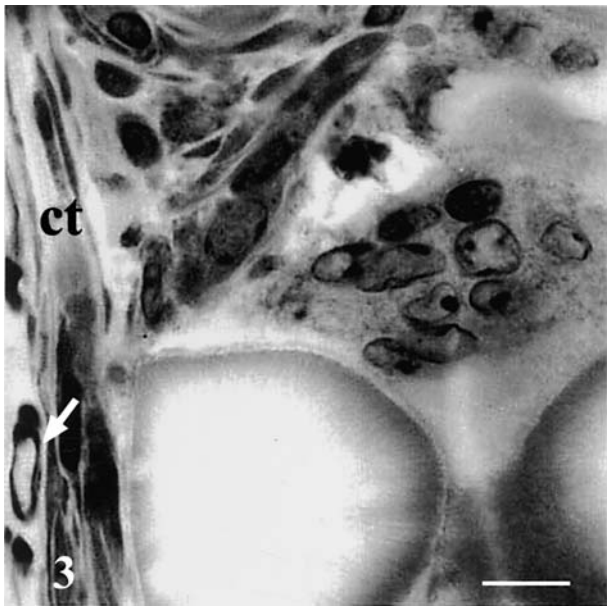

Fig. 3 Photomicrograph of PLLA membrane 21 days after implantation showing a multinucleated giant cell among polymer fragments. Note the capsule of dense connective tissue (ct) and blood vessels (arrow). Bar: 25 μm.

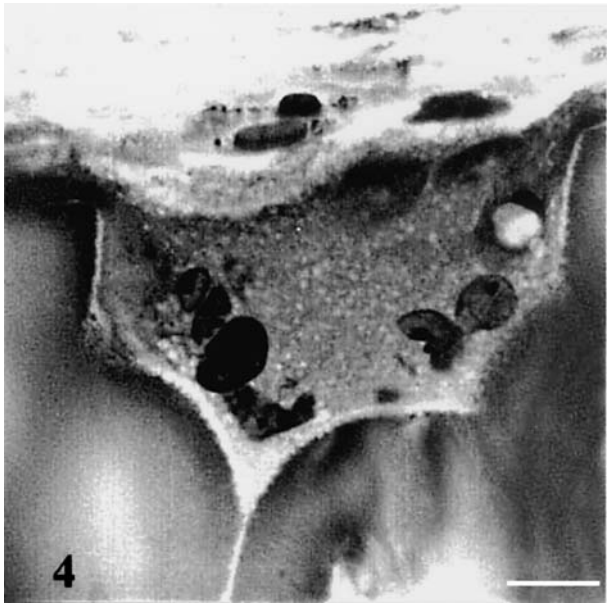

Fig. 4 Photomicrograph of PLLA membrane 60 days after implantation showing a multinucleated giant cell among the polymer fragments. Bar: 25 μm.

By 90 days, the polymer was highly fragmented and was invaded by long extensions of connective tissue (Fig. 5). An average of 4.64 ± 3.60 foreign body giant cells was seen per microscopic field, with 9.60 ± 9.90 nuclei per cell (Table 1).

By 180 days after implantation, the capsule of connective tissue sent ramifications into the membrane, dividing it into

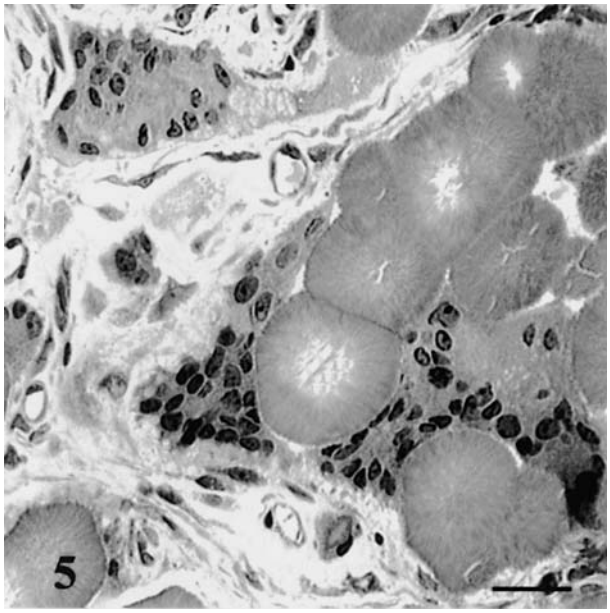

Fig. 5 Photomicrograph of PLLA membrane 90 days after implantation showing multinucleated giant cells surrounding degraded polymeric units. Bar: 50 μm.

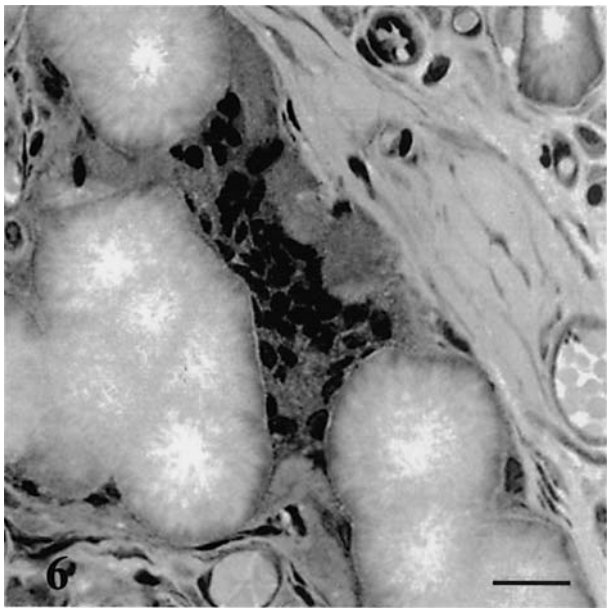

Fig. 6 Photomicrograph of PLLA membrane 180 days after implantation showing a multinucleated giant cell among polymeric fragments invaded by connective tissue. Bar: 25 μm.

smaller fragments. These fragments were surrounded by a thin network of connective tissue. Giant cells were present and contained many nuclei (Fig. 6). The polymer fragments were surrounded by very vascularized connective tissue, and large cells (probably macrophages) with polymer fragments in their cytoplasm were also observed. An average of 4.66 $\pm$ 3.23 giant cells was seen per microscopic field, with 13.48 $\pm$ 16.80 nuclei per cell (Table 1).

Statistical analysis of the number of giant cells and their nuclei

The Kruskal-Wallis test showed that the number of multinucleated giant cells increased significantly from the seventh day to the fourteenth day and from the fourteenth day to the twenty-eighth day after implantation. From the twenty-eighth day to the sixtieth day, there was a significant decrease in the number of giant cells. By the ninetieth day, the number of multinucleated giant cells had increased again statistically significantly and was maintained until the end of the study.

The number of nuclei within these cells increased significantly after ninety days compared to the samples obtained after seven days of implantation and then also increased significantly up to the end of the study.

Discussion

As shown here, within seven days of implantation, the PLLA membrane was surrounded by a fibrous capsule similar to that described by Spector and Tong-Li [18], although the accompanying inflammatory response was less intense than in their study. The membrane pores apparently contributed to the invasion of the implants by tissue elements.

Polymorphonuclear leukocytes, monocytes, macrophages and foreign body giant cells play a central role in the foreign body and immune inflammatory responses that affect the biostability, biocompatibility and effectiveness of the implant [14, 19]. The analysis of samples obtained two days after membrane implantation revealed an inflammatory response with many neutrophils but few eosinophils. Surgical trauma causes an acute inflammatory reaction that can last up to seven days after implantation. This acute inflammatory reaction is then replaced by a reaction to the implant [20, 21].

Mainil-Varlet [9] observed the formation of a capsule consisting mainly of collagen fibers, fibroblasts, fibrocytes and capillaries one month after the implantation of dense pins of PLLA in sheep. Mononuclear cells (macrophages and monocytes) were arranged in direct contact with the surface of the pin. After three months, polymorphonuclear cells were still observed, in addition to mononuclear cells. After six months, the number of phagocytic cells (neutrophils, monocytes and macrophages) around the implant increased, indicating that an acute inflammatory process was still present. One year after the implantation, the capsule consisted mainly of mature collagen fibers and fewer cells.

Solheim *et al*. [22] reported that poly (DL-lactic acid) without plasticizer provoked a chronic inflammatory response involving multinucleated giant cells, macrophages with phagocytosed material and fibroblast proliferation. Pistner *et al*. [2] observed the presence of macrophages and giant cells only during the first weeks after implantation of

PLLA in the subcutaneous tissue of mice. A thin fibrous layer of connective tissue eventually formed and the number of cells decreased.

The presence of giant cells observed in our samples from the seventh day after implantation onwards has also been reported by others [5, 23, 24], but there has been no quantitative analysis of the changes in these cells over time.

The decrease in the number of multinucleated giant cells seen in the samples obtained 60 days after implantation probably reflected the difficulty in preserving the membrane during sectioning of the tissue.

Beumer *et al*. [5] studied double layered degradable implants of poly(ethylene oxide-co-butylene terephthlate) and PLLA without plasticizer and observed that macrophages and multinucleated giant cells containing polymer fragments occurred at implant-tissue interface by the thirteenth week after implantation.

In agreement with other studies [5, 21, 25], histological analysis of the area of the implant showed the formation of vascularized fibrous tissue with the presence of collagen, indicating the repair of tissues damaged during membrane implantation.

Studies of PLLA membranes without the addition of plasticizer [26] have reported the formation of neoplasms in the implantation site. This response may be related to the constant mechanical irritation by the polymer at the site of implantation. In our study, no neoplasms were observed during the 180 days after implantation. Probably the plasticizer addition reduced the rigidity of the polymer and the degradation time, thereby decreasing the chances of neoplasms in the area of the implant [8].

DeFIFE *et al*. [27] demonstrated that the closely related cytokines interleukin 4 (IL-4) and IL-13 can each induce macrophage fusion and foreign body giant cell (FBGC) formation *in vitro* [28, 29] and, further, that IL-4 may participate in the formation of these cells on biomaterials *in vivo* [30], both via a macrophage mannose receptor-mediated pathway.

In conclusion, our results show that PLLA membranes containing 7% of plasticizer do not provoke an exaggerated inflammatory reaction, nor do they interfere with tissue regeneration.

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References

1. K. A. ATHANASIOU, G. G. NIEDERAUER and C. M. AGRAWAL, *Biomaterials* **17** (1996) 93.
2. H. PISTNER, R. GUTWALD, R. ORDUNG, J. REUTHER and J. MÜHLING, *Biomaterials* **14** (1992) 671.
3. J. M. BRADY, D. E. CUTRIGHT, R. A. MILLER and G. C. BARRISTONE, *J. Biomed. Mat. Res.* **7** (1973) 155.
4. M. VAN DER ELST, A. R. DIJKEMA, C. P. KLEIN, P. PATKA and H. J. HAARMAN, *Biomaterials* **16** (1995) 103.
5. G. J. BEUMER, C. A. VAN BLITTERSWIJK and M. PONEC, *Biomaterials* **15** (1994) 551.
6. R. M. LUCIANO, in "Síntese, Caracterização e Degradação de Membranas de Poli(ácido lático), um polímero bioabsorvível", Masters dissertation. UNICAMP. 1997.
7. H. A. VON RECUM, R. L. CLEEK and S. G. ESKIN, A. G. MIKOS, *Biomaterials* **16** (1995) 441.
8. D. R. M. SILVA, S. M. N. SCAPIN, P. P. JOAZEIRO, R. M. LUCIANO, E. A. R. DUEK and M. C. ALBERTO-RINCON, *J. Mat. Sci: Mat. Med.* **13** (2002) 327.
9. P. MAINIL-VARLET, S. GOGOLEWSKI and P. NIEUWENHUIS, *J. Mat. Sci.: Mat. Med.* **7** (1996) 713.
10. N. AL-SAFFAR and P. A. REVELL, *J. Orthop. Res.* **18** (2000) 800.
11. S. H. HYLON, K. JAMSHIDI and Y. IKADA, in "Polymers as Biomaterials" (Plenum Press, 1985) p. 51.
12. K. H. LAM, J. M. SCHAKENRAAD, H. ESSELBRUGGE, J. FEIJEN and P. NIEUWENHUIS, *J. Biomed. Mat. Res.* **27** (1993) 1569.
13. J. M. SCHAKENRAAD, M. J. HARDONK, J. FEIJEN, I. MOLENAAR and P. NIEUWENHUIS, *J. Biomed. Mat. Res.* **24** (1990) 529.
14. W. J. KAO, D. LEE, *Biomaterials* **22** (2001) 2901.
15. A. K. MCNALLY and J. M. ANDERSON, *Am. J. Pathol.* **160** (2002) 621.
16. A. GASSER and J. MOST, *Infect. Immun.* **67** (1999) 395.
17. R. M. LUCIANO, in "Proceedings of the European Medical & Biological Engineering Conference", Vienna, November 1999, edited by P. Peregrius (Published for International Federation for Medical & Biological Engineering, 1999) p. 214.
18. M. C. C. SPECTOR and X. TONG-LI, *Crit. Rev. Biocomp.* **5** (1989) 269.
19. W. J. KAO, *Biomaterials* **20** (1999) 2213.
20. G. J. BEUMER, C. A. van BLITTERSWIJK and M. PONEC, *J. Biomed. Mat. Res.* **28** (1994) 545.
21. J. E. BERGSMA, F. R. ROZEMA, R. R. BOS, G. BOERING, W. C. DE BRUIJN and A. J. PENNINGS, *J. Biomed. Mat. Res.* **29** (1995) 173.
22. E. SOLHEIM, B. SUDMANN, G. BANG and E. SUDMANN, *J. Biomed. Mat. Res.* **49** (2000) 257.
23. D. R. JORDAN, S. BROWNSTEIN, S. GILBERG, B. MATTHEW, L. MAWN and L. KHOURI, *Ophthal. Plast. Reconstr. Surg*. **18** (2002) 342.
24. T. G. van TIENEN, R. G. HEIJKANTS, P. BUMA, J. H. de GROOT, A. J. PENNINGS and R. P. VETH, *Biomaterials* **23** (2002) 1731.
25. J. M. BRADY, D. E. CUTRIGHT, R. A. MILLER and G. C. BARRISTONE, *J. Biomed. Mat. Res.* **7** (1973) 155.
26. S. NAKAMURA, S. NINOMIYA, Y. TAKATORI, S. MORIMOTO, I. KUSABA and T. KUROKAWA, *Acta Orthop. Scand.* **64** (1993) 301.
27. K.M. DEFIFE, C.R. JENNEY, E. COLTON and J.M. ANDERSON, *FASEB J.* **13** (1999) 823.
28. A.K. MCNALLY and J.M. ANDERSON, *Am. J. Pathol*. **147** (1995) 1487.
29. K.M. DEFIFE, C.R. JENNEY, A.K. MCNALLY, E. COLTON and J.M. ANDERSON, *J. Immunol.* **158** (1997) 3385.
30. W.J. KAO, A.K. MCNALLY, A. HILTNER and J.M. ANDERSON, *J. Biomed. Mat. Res.* **29** (1995) 1267.