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b. Maksaja: KYS
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i. Tekijä: Ashammakhi N, Papp A, Sayed R, Ruuskanen M, Kallioinen M, Kellomaki M, Waris T, Seppala J, Tormala P.
j. Teoksen/artikkelin nimi: Histological evaluation of poly(L-lactide/epsilon-caprolactone) membrane implanted subcutaneously in rats.
k. Lehti/sarja: Ann Chir Gynaecol
m. Vuosi: 1999
n. Volyymi: 88
o. Numero: 4
p. Sivut: 313-7
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Ma 05.kesä 2006 10:17:10 **kultamaa - Otettu**

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SNE LSALI LEHTI

t. Pirkko

Ma 05.kesä 2006 10:25:48 **kultamaa - Tila muutettu arvosta uusi arvoon avoin**

Ma 05.kesä 2006 10:26:08 **kultamaa - Ottamaton**

Ma 05.kesä 2006 10:26:09 **kultamaa - Queue muutettu arvosta kys-kirjasto arvoon kopiopalvelu**

Annales Chirurgiae et Gynaecologiae 88: 313–317, 1999

HISTOLOGICAL EVALUATION OF POLY(L-LACTIDE / EPSILON-CAPROLACTONE) MEMBRANE IMPLANTED SUBCUTANEOUSLY IN RATS.

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ABSTRACT

Background: **Poly(L-lactide/ε-caprolactone) [P(L-LA/ε-CL)] 50/50 membranes is an absorbable membarane which has been developed recently for possible use in tissue engineering.**
Aims: **To look at histological tissue reactions to the membrane and its behaviour upon its *in vivo* implantation in rats.**

Material and methods: **P(L-LA/e-CL) 50/50 membranes, 0.4 mm thick, were implanted subcutaneously in the dorsal neck of 20 Wistar rats. The rats were followed-up for 1, 3, 6, 12 and 28 months. After sacrifice, subcutaneous tissues with implants were taken as specimens, inspected for any gross abnormality and histological examination was carried out.**

Results: **No significant macroscopic changes were noticed except for two implants that were grossly rounded. Histologically, the membranes had undergone cracking, fragmentation and progressive degradation. Cracks appeared initially at the periphery of the implant. Large cracks progressed mainly in longitudinal planes. Cracks occurred in the middle of implants and led to membrane bending in some cases. "Neomembrane" formation, comprised of fibrous tissue and the implant, was seen. The foreign-body reaction to the membrane involved macrophages and foreign-body giant cells. In one case an acute type of polymorphonuclear cell reaction was observed and in a second case a predominantly lymphocytic reaction was seen at three months. They were thought to be the results of infection. No other adverse effects were seen.**

Conclusions: **P(L-LA/e-CL) 50/50 membrane was found to be biocompatible when implanted subcutaneously in rats. It was degraded to a great extent but not completely in 28 months.**

Key words: Lactide; caprolactone; biodegradable; neomembrane

INTRODUCTION

Poly (L-lactide / epsilon-caprolactone) [P(L-LA/ε-CL) 50/50] is a biodegradable synthetic copolymer of lactic acid (LA) and ε-caprolactone (ε-CL). Degradation of the polymer is known to proceed in at least two stages. First there is non-enzymatic, random hydrolytic ester cleavage. In the second stage, weight loss of the polymer occurs because of diffusion of oligomers from the material and the polymers become prone to enzymatic erosion and phagocytosis (1).

P(L-LA/ε-CL) membranes, with variable LA/CL ratios, have been studied mainly in connection with guided nerve regeneration. Biocompatibility has been proved and successful nerve regeneration achieved (2–8).

Tissue engineering is a growing field in which biomaterials, that act as scaffolds and tissues or cells are used to develop biological substitutes. The purpose of this study was to examine histologically the behaviour of a newly manufactured P(L-LA/ε-CL) 50/50 membrane implanted subcutaneously in rats and to examine the reaction of the surrounding tissues as regards its possible use in tissue engineering.

MATERIALS AND METHODS

IMPLANTS

The copolymer of L-lactic acid and ε-caprolactone P(L-LA/ε-CL) used in this work was polymerised in Helsinki (9, 10). The material was used as polymerised and the initial monomer ratio was 50/50. The residual monomer content of the polymer was approximately 5 % by weight, and in principle only the ε-CL monomer was present in the polymer.

Films with a nominal thickness of 0.4 mm were compression-moulded in a laboratory scale hydraulic press at 150°C, at Tampere University of Technology. The raw polymer films were cut into pieces of 10 x 30 mm. Special care was taken in cutting so that the edges of these pieces were smooth and no stress-concentrating points occurred. They were sterilised by γ-irradiation, using a minimum dose of 2.5 Mrad. The initial average molecular weight of the samples after γ-irradiation was 60 000 Da.

ANIMALS, EXPERIMENTAL AND ANALYSIS METHODS

Twenty two-week-old male rats were operated on. The animals were anaesthetised using Hyponorm-Midazolam (1:1 ratio, diluted in saline, 0.15–0.20 mg/100g) s.c. injections. The dorsal side of the neck was scrubbed with antiseptic solution. A 3-cm-long midline incision was made between the scapulae. A subcutaneous pocket was prepared by blunt dissection on both sides of the midline incision. One P(L-LA/ε-CL) membrane was placed inside each pocket. Each membrane was sutured to the dorsal fascia using one stitch on either side with 4.0 Dermalon, to serve as a marker. The skin and subcutaneous tissue were closed in one layer using 4.0 Dermalon sutures.

Post-operatively, attention was directed towards the general health of the rats and any external gross changes. Three rats were sacrificed after one month, 5 after three months, 5 after six months, 4 after twelve months and 3 after 28 months. Sacrifice was carried out using a carbon monoxide chamber. After sacrifice, the membranes were dissected along with the surrounding fibrous tissue capsule and taken as specimens. During retrieval of the specimens, the implants and implant beds were inspected for any gross abnormality. The specimens were immersed in formalin and transported to the laboratory, which received them the next day.

The specimens were processed, embedded in paraffin and cut into sections of 4 micrometers thick, using at least three different areas of each specimen. Histological examination was carried out after staining with haematoxylin and eosin, Alcian blue, toluidine blue and Verhoeff's stains. Membrane degradation and resulting surrounding tissue reactions were evaluated using light microscopy.

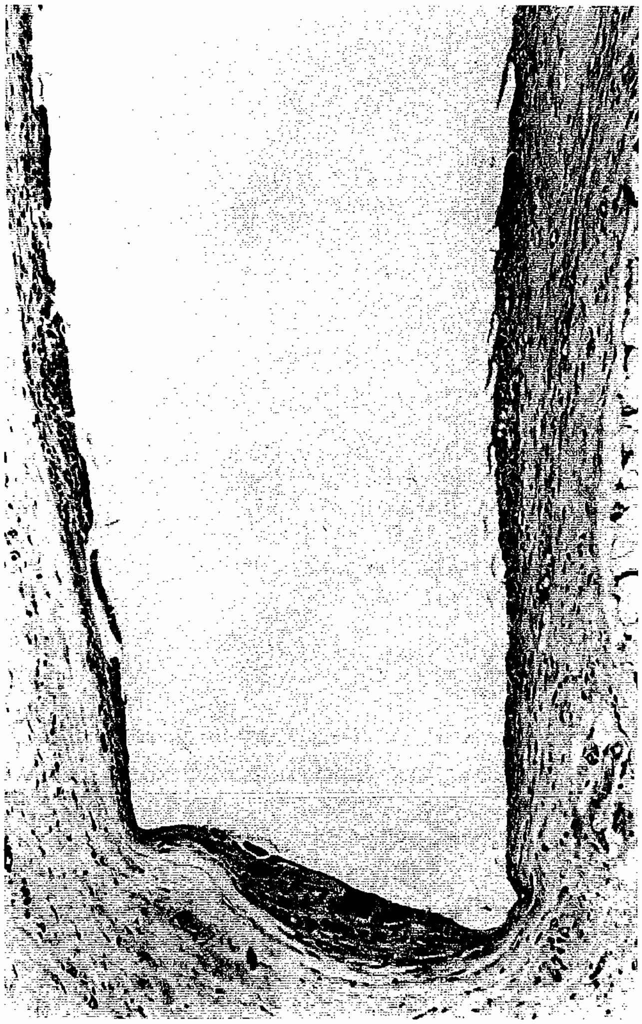

Fig. 1. Photomicrograph of a subcutaneously implanted P(L-LA/ε-CL) 50/50 membrane one month postoperatively. The implant is surrounded by a fibrous tissue capsule. (Haematoxylin & eosin, original magnification x 100).

RESULTS

MACROSCOPIC

The postoperative period was uneventful. The wounds healed well. On retrieval of the specimens, the membranes were seen to be wrapped in fibrous tissue. The whole composite could be dissected free from the host subcutaneous tissue bed. On checking the relationship between the fibrous capsule and the implant, it was found to be easily detachable from the implant in the initial follow-up times of 1–6 months. In the three-month group, membranes from two rats were found to resemble cystic balls; otherwise, no gross abnormalities were noticed.

MICROSCOPIC

After one month:

The membrane material was still seen to be a single intact piece. It was surrounded by a fibrous tissue

Fig. 2. Photomicrograph of P(L-LA/ε-CL) 50/50 membrane six months postoperatively. The implant is surrounded by a fibrous tissue capsule. Cracks now extend deeply into the implant. Note the longitudinal direction of the cracks. There is also a cellular reaction involving fibroblasts, macrophages and giant cells around the implant and between implant fragments. (Haematoxylin & eosin, original magnification x 100).

Fig. 3. Photograph of P(L-LA/ε-CL) 50/50 membrane 28 months postoperatively. The membrane has been degraded to smaller fragments. Cellular elements can be seen inside the implant, reflecting a foreign-body reaction and progressive degradation and absorption. (Haematoxylin & eosin, original magnification x 100).

capsule which contained fibroblasts, macrophages and foreign-body reaction giant cells (Fig. 1).

After three months:

The membrane was seen to be a single piece with a few small cracks at the periphery. A large crack in the middle of the membrane was noticed in most cases. A capsule of fibrous tissue could be seen around the implant. This capsule contained macrophages and foreign-body reaction giant cells. In one specimen, the inflammatory reaction mainly involved polymorphonuclear cells and in another specimen, lymphocytes. In these two cases, the composite of implant and fibrous inflammatory tissue was rounded in shape. This feature was reported on gross examination on retrieval of these specimens.

After six months:

Cracks in the membrane were larger and more numerous than earlier. Cracks occurred mainly in the longitudinal axis of the implant, leading to almost complete division of the membrane. The implant was observed to take stain more than in earlier follow-up periods. Fibrous tissue was seen filling the spaces between the fragments. Collagen in the capsule was more polarised and mature than in earlier follow-up periods (Fig. 2).

After twelve months:

The implant had undergone progressive fragmentation. Implant bending was seen in two specimens. In one of these specimens the bending was more acute than in the other. Implant fragments were smaller in size than earlier. Individual fragments were surrounded by fibrous tissue, macrophages and giant cells.

After twenty-eight months:

Only small fragments of the implant were seen inside an inflammatory fibrous tissue capsule. Macrophages and giant cells were abundant in between these fragments. Blood vessels in the capsule were also prominent (Fig. 3).

DISCUSSION

At early stages, the fibrous tissue capsule was easily separable from the implant. This is probably because the newly developed fibrous tissue capsule had not yet extended into the membrane material.

In the three-month group, the membranes were rounded in two cases. In one, there was an acute polymorphonuclear type of inflammation. In the other case a lymphocytic reaction was seen. These may be the result of infection. Membrane bending may be explained by the presence of large cracks in the centre of the membranes. Myofibroblasts, which are known to take part in wound healing, had probably pulled in the edges of these membranes. At six months, the cracks were more clearly in the longitudinal axis of the implant, leading to almost complete division of the membrane into separate pieces. This is an advantage in the sense that deformation of the implant occurred parallel to tissue planes. This may reduce the risk of particle dispersion and postoperative complications.

The fibrous tissue encapsulation of foreign-body implants was again observed in this study. It is well known that these implants induce fibroplasia or so-called "neomembrane" formation. We have observed neomembrane composite formation earlier and introduced the term (11). Neomembranes mature with time, as evidenced by the collagen arrangement and polarisation. Angiogenesis was observed and this is thought to be necessary to support neomembrane metabolism. Macrophages are thought to be responsible for ultimate digestion and clearing of decomposing polymers such as PGA. This was considered to be the case by Päivärinta et al. (12), who observed macrophages around PGA screws implanted in rabbit cancellous bone. den Dunnen et al. (2) have studied various P(LA/ε-CL) nerve guides with wall thicknesses of 0.34–0.68 mm. After 2 months, all types of nerve guide had changed from a transparent to an opaque and swollen state, and they had lost their strength. The foreign-body reaction was characterised by the presence of giant cells and fibroblasts surrounding the degrading nerve guide. In our study, we observed that the implants were more opaque from six months onwards than at earlier follow-up times. This is probably due to changes in the internal structure of the implant, hydration and degradation.

Subcutaneous degradation of porous 50/50 copoly(LLA/ε-CL) 0.15 mm-thick films has been studied in rats (13). The copolymer phase was observed to separate into a crystalline phase containing mainly L-lactide and an amorphous phase containing mainly ε-CL. Transparent implants became opaque following implantation. By 56 weeks, the implants had fragmented but were not completely absorbed. With a longer follow-up time of 28 months (present study), implant fragments were still seen. After implantation, cracks develop and are thought to enable water to reach into deeper parts of the implant. This leads to hydrolytic scission of the polymer and subsequent cellular degradation of smaller particles follows. Progressive fragmentation of the membrane was observed and the size of individual fragments became smaller throughout the follow-up time, leading to an overall decrease in the gross size of the implant.

den Dunnen et al. (3) have evaluated the degradation of P(DLLA/ε-CL) (PLA85/CL15) bars implanted subcutaneously in rats. It was observed that the material degraded completely within 12 months. A foreign-body reaction was also observed. Poly(LLA-co-6-CL) nerve conduits (with a wall thickness of 175 microns) have been studied and found to be still undamaged 30 days after implantation, but progressive signs of degradation appeared at 90 and 180 days (4, 6). These conduits have been used to bridge a gap of 10 mm in the sciatic nerves of Wistar rats, with effective nerve regeneration. In the present study, P(L-LA/ε-CL) was still seen 28 months after implantation, which is probably due to different chemical composition, thickness and manufacturing technique.

P(L-LA/ε-CL) membrane is somewhat soft in character and it is possible to fold it to a desired shape, which makes it easy to handle and apply in surgery. However, stabilisation of the membrane, e.g. by suturing, may be necessary to hold it in place when surgical applications are undertaken.

The degradation and absorption process appears to be prolonged. It probably goes far beyond the time required for guided tissue regeneration. Further modification and adjustment of the chemical and physical properties of the membrane may be necessary to achieve earlier absorption. However, this should be investigated in specific application studies.

CONCLUSIONS

In this study, P(L-LA/ε-CL) 50/50 membrane implanted subcutaneously in rats was degraded to a great extent but not completely after 28 months. Cracks appeared initially at the periphery of the implant and progressed mainly in longitudinal planes. P(L-LA/ε-CL) membrane implantation led to "neomembrane" formation which comprised fibrous tissue and implant fragments. The foreign-body reaction to the membrane involved macrophages and foreign-body giant cells except in one case where it was a polymorphonuclear and in another case a lymphocytic reaction, most probably as a result of infection. No other adverse reactions were observed. This makes the membrane of potential use in tissue engineering.

ACKNOWLEDGEMENTS

This study is part of the Biomedicine and Health Programme funded by the European Commission. These funds are greatly appreciated. Research funds received from the Technology Development Centre in Finland (TeKes) are also greatly appreciated.

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Received: December 14, 1998
Accepted: April 19, 1999

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