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Preventing postoperative intraperitoneal adhesion formation with Polyactive™, a degradable copolymer acting as a barrier

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In this study a degradable barrier, composed of a poly(ethyleneglycol) and poly(butyleneterephthalate) copolymer (1000 Polyactive™ 55/45), was investigated for the prevention of postoperative adhesion formation. Three different designs of polymer films were used in a standardized rat adhesion model in order to investigate which barrier form would be most efficacious in reducing adhesion formation. Neither non-porous nor porous film reduced the adhesion formation as compared with control experiments. A bilayered film, however, composed of a porous underlayer and a non-porous toplayer, reduced the adhesion formation significantly compared with control animals without a barrier. In the case of adhesion formation with this bilayered film the adhesions were limited to areas that were macroscopically not covered, either due to fractures in or detachment of the barrier. Subsequently a more malleable porous/dense film was designed which did not fracture and reduced the adhesion formation significantly compared with animals treated with Interceed™, even though some barriers detached as in the first experiment. The porous/dense Polyactive™ barrier seems to be a promising adjuvant in the reduction of postoperative adhesion formation, although further research is needed to accomplish better attachment of the barrier.

1. Introduction

Several studies report the occurrence of postoperative intra-abdominal adhesions in 50 to 95% of women undergoing gynecological surgery [1, 2]. Bowel obstruction and/or chronic pelvic pain are common complications due to this adhesion formation. Additionally, adhesions may cause a problem in the gynecological patient because of their interference with fertility [3, 4]. Owing to these problems a wide variety of adjunctive treatments to prevent the formation and reformation of adhesions has been proposed. However, the results achieved with these different treatments are rather inconsistent [5–7]. One of the modalities that has been studied extensively is the so-called barrier method. With the barrier technique, surgically traumatized surfaces are kept covered during mesothelial regeneration thus preventing adherence of adjacent structures and reducing adhesion formation. The most commonly used barrier materials clinically are oxidized, regenerated cellulose (Interceed™) [8, 9] and expanded polytetrafluoroethylene (Gore-Tex Surgical Membrane™) [11–13]. Although effective in reducing adhesion formation, both materials have limitations for usage in the clinical setting. While polytetrafluoroethylene is non-degradable and requires a second operation for removal, degradable cellulose can be applied only on dry, non-bleeding surfaces [14].

We therefore sought a new, degradable material that would be able to prevent adhesions, both on non-bleeding and bleeding surfaces. Polyactive™, a copolymer based on poly(ethyleneglycol) and poly(butyleneterephthalate) with degradation characteristics that can be altered by varying the composition of the two components [15–19], was investigated in three different structures to study its efficacy in reducing postoperative adhesion formation. A rat paradigm was used as the standard adhesion model [20] to study a dense film, a completely porous, perforated film and a bilayered combination of a porous and dense film. Sham-operated and Interceed™-treated animals served as controls.

2. Materials and methods

2.1. Surgical techniques

A total of 41 female Wistar rats (Harlan CPB, Zeist, The Netherlands) of reproductive age, weighing 180–200 g, were used. The rats were anesthetized with ether after which the abdomen was shaved and iodine prepped.

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A standard adhesion model described earlier [20] consisted of the excision of a parietal peritoneal defect of 1.5 by 1.2 cm, which was closed with three Vicryl (polyglactin 910) (Ethicon) 5–0 sutures in 2 = 2 knots. The uterine horn was clamped three times with artery forceps, for 10 s, and sutured both proximally and distally of the defect with Prolene (polypropylene) (Ethicon) 6–0 sutures [20]. All barriers were randomly assigned to the peritoneal defects and applied without suturing (Fig. 1). The porous/dense barriers were placed with the porous side facing the peritoneal defect. Control animals were subjected to a similar procedure but without a barrier being applied. No attempt at hemostasis was made.

2.2. Barrier films and experimental design

Barrier films made of 1000 polyactive™ (HC-Implants B.V., Leiden, The Netherlands) were prepared by a solvent casting method. The 1000 Polyactive™ 55/45 was composed of 55 wt % poly(ethyleneglycol), molecular weight 1000, and 45 wt % poly(butyleneterephthalate). Three solutions of a 1:10 ratio of the 1000 Polyactive™ in chloroform were made. To two of these solutions sodium citrate particles were added for the creation of pores. These particles measured 150–212 μm for the first porous/dense film (PD1) and 106–150 μm for the second porous/dense film (PD2). The third Polyactive™ solution without sodium citrate particles was used to cast the dense layers and films.

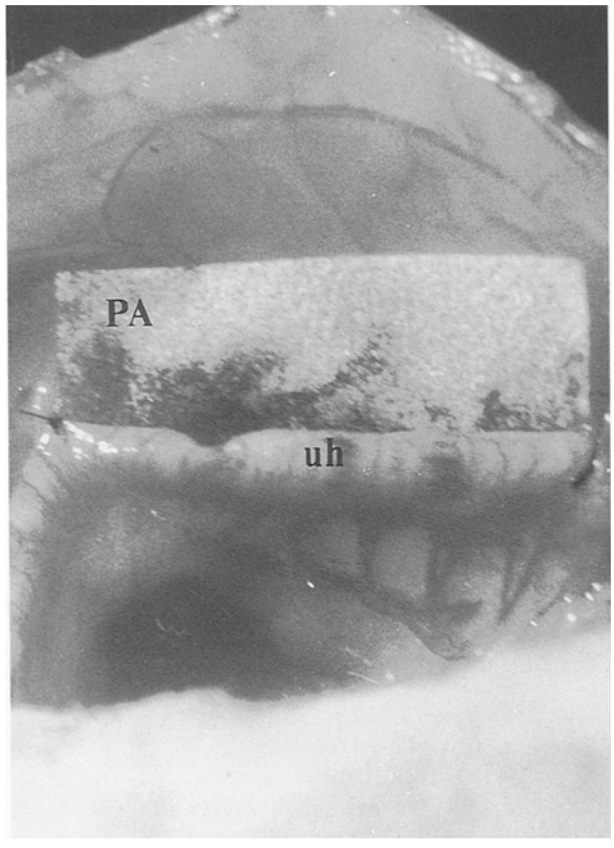

Figure 1 A PD2*-barrier as positioned at the peritoneal defect after completion of the operative procedure. UH = uterine horn; PA = Polyactive barrier (magnification 4×).

Two porous layers were cast and after being allowed to dry, were followed by a dense top layer. Thus two bilayered films with a final thickness of 250 μm (PD1), respectively, 205 μm (PD2) were produced. Subsequently two dense films were cast (final thickness 42 μm, respectively, 70 μm) of which the latter (P) was perforated with a punch (diameter $25\frac{5}{8}$) on each square millimetre. After casting and chloroform evaporation the films were immersed in distilled water and air dried. Subsequently barriers, measuring 2.0 by 1.5 cm, were cut from the films, vacuum sealed and gamma-sterilized (Gammaster B.V., Ede, The Netherlands). Interceed™ was generously supplied by Johnson and Johnson Med. (Amersfoort, The Netherlands).

In the first experiment a total of 52 peritoneal defects was randomly assigned to five different groups; PD1, P, D, Interceed™ and sham-operated animals. In the second experiment a total of 30 peritoneal defects was randomly assigned to three different treatment groups; PD2, Interceed™ and sham-operated animals. (For experimental design see Table I.)

2.3. Scoring of the adhesions

Two weeks postoperatively the rats were sacrificed and the adhesions were scored according to their extent.

As previously described [20] the area to be scored was divided through the three vicryl sutures with which the peritoneal defect was closed, into eight areas of 12.5%. An adhesion score of 100% could thus be achieved maximally.

2.4. Microscopic techniques

After the macroscopic scoring was performed the areas were dissected and fixed overnight by immersion in 1.5% glutaraldehyde in 0.14 M sodium cacodylate buffer at 4 °C (pH 7.4). After dehydration through a graded ethanol series the specimens were critical point dried and sputter-coated with gold for evaluation in a Philips SEM 525 M scanning electron microscope. For light microscopy the specimens were dehydrated and embedded in glycol methacrylate and cut into 2 μm thick sections on a Reichert Jung 2050 microtome. The sections were stained with toluidine Blue or Giemsa.

TABLE I Experimental design of the two experiments

Experiment I (n = 52)	Experiment II (n = 30)
– PA D dense films (n = 14)	– PA PD2 porous/dense film (n = 10) 205 μ, pores 106–150 μ
P perforated films (n = 14)	
PD porous/dense films (n = 14) 250 μ, pores 150–212 μ	
– Interceed (n = 4)	– Interceed (n = 10)
– Sham-operated (n = 6)	– Sham-operated (n = 10)

PA = Polyactive™.

2.5. Statistical analysis

Statistical analysis was performed using the one-way analysis of variance (Anova) and least-significant-difference (LSD) multiple range test. Statistical significance was defined as $p < 0.05$. Data were expressed per peritoneal defect as means ± SD.

3. Results

3.1. Macroscopic observations

In all animals that were studied adhesion formation was confined to the peritoneal defects. One rat died perioperatively and one rat postoperatively during anesthesia. No other pathological conditions, i.e. bowel obstruction, peritonitis or abscesses were found.

At postoperative evaluation the control animals that were operated without appliance of a barrier showed an average adhesion percentage of 79.2 (± 23.3) (first experiment), respectively, 81.3 (± 15.9) (second experiment) per peritoneal defect (Figs 2 and 3).

The Interceed™-treated peritoneal defects scored an average adhesion percentage of 75.0 (± 33.9) (first experiment), respectively, 82.5 (± 30.7) (second experiment). In all cases Interceed™ was still at its original location, remaining as a brownish structure covered with adhesions.

All dense Polyactive™ barriers of the first experiment had detached from the peritoneal defects. The average adhesion percentage in this treatment group was 73.1 (± 19.7).

In contrast to the dense barriers all perforated barriers were still attached to the peritoneal defects and an average adhesion percentage of 73.1 (± 34.6) was found with this group. The porous/dense Polyactive™ barriers showed an average adhesion percentage of 43.8 (± 39.7) (first experiment, PD1) and 52.5 (± 43.7) (second experiment, PD2).

In the PD1 group, four of the 12 films had detached from the peritoneal defect and adhesion formation had occurred on these uncovered defects. In addition to adhesions on these uncovered defects, adhesions formed in between fractured areas of the PD1-barriers. No adhesion formation was found on the areas that were covered by intact parts of the PD1-barriers.

The PD2-barriers showed no fractures macroscopically. Adhesions formed only on uncovered defects (five out of ten).

When the scores of the detached PD-barriers were excluded from the overall score the average adhesion percentages of the PD-covered defects were 39.1 (± 39.7) for the PD1-barrier (PD1* in Fig. 2) and 15.0 (± 22.4) for the PD2-barrier (PD2* in Fig. 3). All porous/dense barriers that remained at the peritoneal

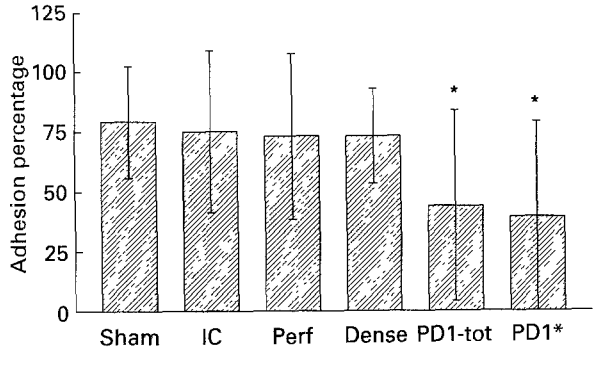

Figure 2 Adhesion formation with the different barrier designs of the first experiment. Sham = sham-operated animals, IC = Interceed, Perf = perforated barriers, PD1-tot = adhesion formation at all PD1-treated defects, PD1* = adhesion formation at the defects that were macroscopically covered with PD1-barriers. PD1-total differed significantly from sham-operated animals. PD1* from dense, perforated and sham-operated animals ($p < 0.05$).

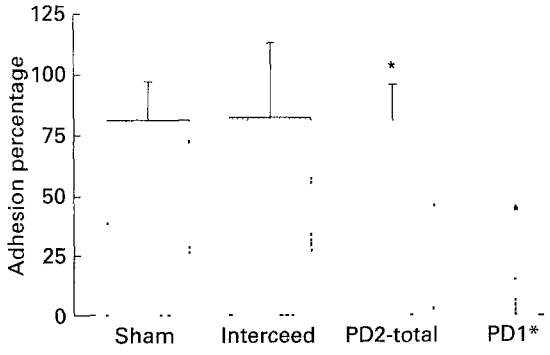

Figure 3 Adhesion formation with the PD2-barrier. Sham = sham-operated animals, PD2-total = adhesion formation at all PD2-treated defects. PD2* = adhesion formation at the defects that were macroscopically covered with PD2-barriers. PD2-total differed significantly from the Interceed-treated group, PD2* both from Interceed and sham-operated animals.

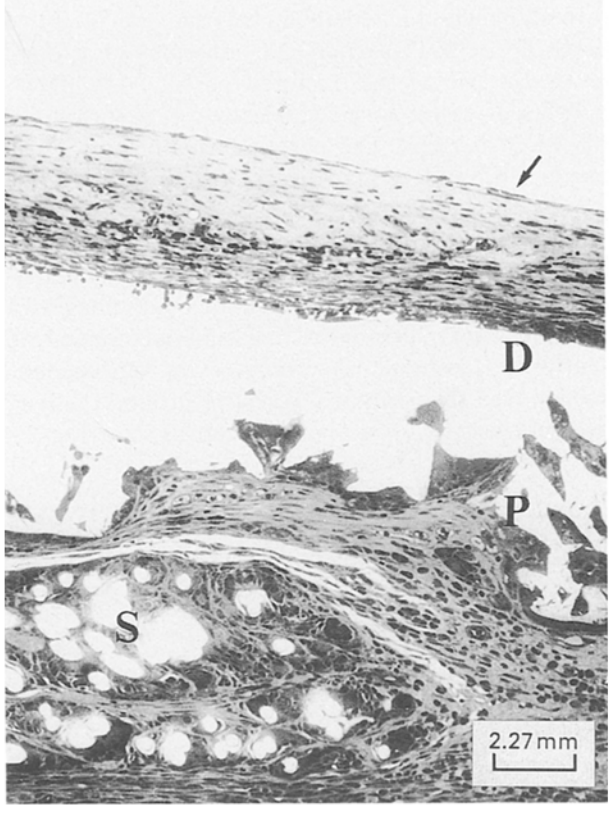

Figure 4 Light micrograph (stain toluidine blue) showing the peritoneal defect being covered by a PD2*-barrier. The barrier itself is covered by a layer of fibrous tissue and confluent mesothelium (arrow). No adhesion formation is seen at the surface of the barrier. P = porous layer; D = dense layer; S = Vicryl suture with which the peritoneal defect is closed.

defect were covered by a transparent layer of tissue that resembled the normal peritoneum.

The films that were detached could generally be retrieved either in the omentum, pelvic fat body or mesosalpinx of the uterine horn. Two PD-barriers were found attached to the liver. No adhesions to the detached films occurred.

3.2. Microscopic observations

3.2.1. Light and scanning electron microscopy

Only the sham-operated animals and the defects that were still covered with PD-barriers (PD1* and PD2*) were evaluated microscopically. Light microscopy showed that the peritoneal defects in both PD-treated groups were covered by the Polyactive™-barriers (Fig. 4). The barriers showed fragmentation and the dense layer was completely covered by fibrous tissue, consisting of collagen and fibroblasts, and confluent flat mesothelium (arrow, Fig. 4). Adhesion formation was seen only in between the fractured areas of the PD1*-barriers. No adhesions were found at the surface of the fractured parts of the PD1*-barriers or at the macroscopically intact surfaces of the PD2*-barriers. The porous layers of both PD*-barriers showed complete ingrowth of vascularized, fibrous tissue (Fig. 5).

The cellular reaction around the barriers was dominated by phagocytes. Fragments of the barrier material were seen intracellularly within macrophages or giant cells that surrounded the material. Almost no neutrophils, eosinophils or lymphocytes were encountered within the peritoneal defect or around the barriers, nor was tissue oedema or necrosis seen (Fig. 5).

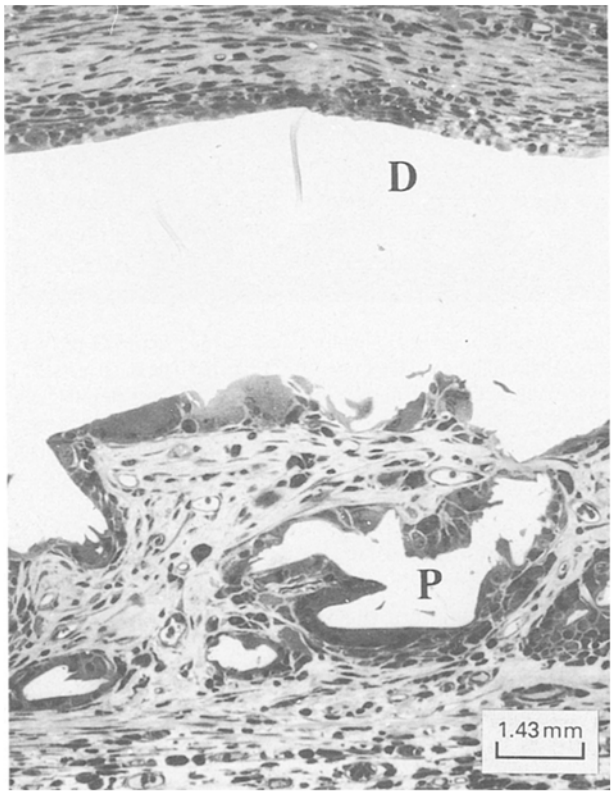

Figure 5 Light micrograph (stain toluidine blue) showing ingrowth of vascularized, fibrous tissue into the pores of a PD2*-barrier. P = porous layer; D = dense layer.

Scanning electron microscopy showed that all PD-barriers that were still attached (Fig. 6) were covered by polygonal mesothelial cells. These mesothelial cells formed a confluent layer and had large numbers of microvilli at their surface (Fig. 7).

4. Discussion

In the present study Polyactive™, a degradable copolymer based on poly(ethyleneglycol) and poly(butyleneterephthalate), was studied for its efficiency in reducing postoperative adhesion formation. Three different types of barrier films were produced to study which design would be the most efficacious in preventing postoperative adhesion formation.

The porous/dense bilayered film possessed the best characteristics for preventing adhesion formation since its structure included two aspects. First, the dense top layer did not allow the passage of tissue components or cells through the barrier [21]. The film thus acts as a mechanical separation between adjoining surfaces, according to the barrier technique. Sec-

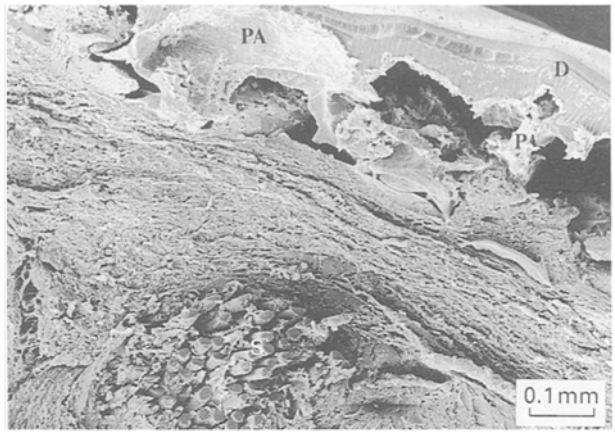

Figure 6 Scanning electron micrograph. As in Fig. 2, a PD2*-barrier is shown, covering the peritoneal defect. No adhesion formation was seen at the surface of the barrier. PA = Polyactive; P = porous layer, D = dense layer; S = Vicryl suture with which the defect is closed.

Figure 7 Scanning electron micrograph showing the surface of a PD2*-barrier that was macroscopically attached to the defect. Confluent mesothelium as shown in this picture covered the PD-barriers completely. Note the abundance of microvilli at the surface of the mesothelial cells

ond, the porous underlayer assured fixation of the Polyactive™ PD-barriers because it allowed ingrowth of surrounding tissue [17]. Both PD-barriers, provided they remained macroscopically intact and adhered well, were indeed highly efficacious in reducing adhesion formation. Since the barriers of the first PD-serie fractured, probably due to brittleness of the material, a more malleable PD-film was produced for the second experiment. In the preparation of these PD2-films smaller sodium citrate particles were used. Additionally, the thickness of both porous and dense layers was reduced, as compared with PD1. The average pore size was restricted by the necessity to obtain ingrowth of tissue [18, 22, 23]. Although these PD2-barriers still detached in five out of the ten cases, none fractured macroscopically. When the barriers remained attached to the peritoneal defect only 15% adhesion formation was found. Peripheral detachment of one PD2-barrier accounted mostly for this remaining percentage since this barrier showed an adhesion percentage of 50. Three of the five remaining PD2-barriers showed no adhesion formation whatsoever while adhesions were present at every defect in the sham-operated groups of both experiments.

All barriers were applied without suturing the material to the abdominal side wall. This approach was based on pilot studies showing adequate adherence of the 1000 Polyactive™ 55/45 films to the peritoneum without suturing. Besides, sutures themselves have been implicated in adhesion formation. The adherence of the Polyactive™ barriers immediately after placement is thought to depend on hydrostatic capillary action caused by the intrinsic porosity and swelling characteristics of the PD-barriers. However, the final detachment in 25% of the PD1-barriers and in 50% of the PD2-barriers is probably due to the fact that swelling of the material was completed, thereby allowing the material to detach, before ingrowth of surrounding tissue is able to accomplish permanent anchorage of the barrier.

None of the dense barriers stayed attached to the defects, which might be explained by the total absence of pores. The perforated barriers, on the contrary, all remained at the peritoneal defects but gave an adhesion percentage similar to the sham-operated and Interceed™-treated animals. This adhesion formation is probably due to the same phenomenon that causes adhesions when Interceed™ is soaked with blood when placed on a bleeding surface [14]. Similar to the situation with the perforated Polyactive™ barriers, cells and blood products are thought to penetrate Interceed™ in this situation, resulting in contact between adjacent structures and subsequent adhesion formation may occur. Our results are comparable with two papers recently published that described a higher, respectively, similar adhesion percentage with Interceed™ as compared with controls when used in a rodent model [13, 24].

Degradation of the PD-barriers could be identified by the presence of microscopic fragmentation of the material and by degradation products sequestered in macrophages and giant cells. As previously described, the PD-barriers evoked a mild foreign body reaction [15–19]. When compared with a sham-operated defect, only the number of macrophages involved was slightly larger with the Polyactive™-treated animals. Since the barriers were covered completely by a regenerated, confluent mesothelial layer, adhesion formation at later stages due to degradation is not anticipated.

It is concluded that a significant reduction in the formation of postoperative adhesions was achieved with porous/dense Polyactive™ barriers. The dense layer functioned as a barrier and allowed mesothelial overgrowth, while the porous layer allowed ingrowth of vascularized, fibrous tissue attaching the barrier permanently. Further investigation is needed to achieve better initial adherence at the original application site of this new, degradable barrier.

References

1. T C M TRIMBOS-KEMPER, J B. TRIMBOS and E V. VAN HALL, *Fertil. Steril.* **43** (1985) 395.
2. G HOLTZ, *ibid.* **41** (1984) 497.
3. V. GOMEL, *ibid.* **39** (1983) 144.
4. W. H PFEFFER, *ibid.* **33** (1980) 245.
5. G S. DIZEREGA and G. D. HODGEN, *Amer. J. Obst. Gyn.* **136** (1980) 173.
6. C J. LEVINSON and K SWOLIN, *Clin. Obst. Gyn.* **23** (1980) 1213.
7. A A LUCIANO, K S HAUSER and J. BENDA, *Amer. J. Obst. Gyn.* **146** (1983) 88.
8. C B. LINSKY, M P. DIAMOND, T. CUNNINGHAM, B. CONSTANTINE, A H DECHERNEY and G. S. DIZEREGA, *J. Reprod. Med* **32** (1987) 17.
9. Adhesion Barrier Study Group Interceed (TC7), *Fertil Steril.* **51** (1989) 933.
10. The Japanese Study Group, *Obst. Gyn.* **58** (1991) 1424.
11. S P. BOYERS, M P DIAMOND and A H DECHERNEY, *Fertil. Steril.* **49** (1988) 1066
12. J M GOLDBERG, A A TOLEDO and D E. MITCHELL, *Obst. Gyn.* **70** (1987) 846.
13. A. F HANEY and E. DOTY, *Fertil. Steril.* **57** (1992) 202.
14. C. B LINSKY, M P DIAMOND, A. H. DECHERNEY, G. S DIZEREGA and T CUNNINGHAM, *Infert.* **11** (1988) 273.
15. J J GROTE, D BAKKER, S C HESSELING and C A VAN BLITTERSWIJK, *Amer. J. Otol.* **12** (1991) 329.
16. D BAKKER, C. A VAN BLITTERSWIJK, S. C. HESSELING, H. K KOERTEN, W. KUIJPERS and J J GROTE, *J. Biomed. Mater. Res.* **24** (1990) 489.
17. D. BAKKER, C A. VAN BLITTERSWIJK, S. C. HESSELING, J. J GROTE and W TH. DAEMS, *Biomaterials* **9** (1988) 14.
18. C A. VAN BLITTERSWIJK, S C. HESSELING, I VAN DEN BRINK, H LEENDERS and I VAN DEN BRINK, in "The bone–biomaterial interface" (University of Toronto Press, 1991) p. 295.
19. D BAKKER, C A VAN BLITTERSWIJK, S. C. HESSELING , W. TH DAEMS and J J. GROTE, *J. Biomed Mater. Res.* **24** (1990) 809.
20. E. A. BAKKUM, C. A VAN BLITTERSWIJK, R. A J DALMEIJER and J B. TRIMBOS, submitted
21. G. J. BEUMER, D. BAKKER, A A. VAN BLITTERSWIJK and M PONEC, accepted in *Clin. Mater*
22. J D BOBYN, G J. WILSON, D. C. MACGREGOR, R M PILLIAR and G C WEATHERLY, *J. Biomed. Mater. Res.* **16** (1982) 571.
23. C. C. CHU and L. WELCH, *ibid.* **19** (1985) 903.
24. K PAGIDAS and T. TULANDI, *Fertil. Steril.* **57** (1992) 1992.

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