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Biodegradation of non-porous films after submucoperiosteal implantation on the palate of Beagle dogs

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The aim of this study is to investigate the *in vivo* behaviour of five different biodegradable films after submucoperiosteal implantation on the palate of Beagle dogs. Non-porous films of poly-(L-lactic) acid (=PLLA), high molecular weight poly-(L-lactic) acid (=HMW-PLLA), poly-(DL-lactic) acid (=PDLLA), poly(ε-caprolactone) (=PCL) and a copolymer of poly hydroxybutyrate 80%–hydroxyvalerate 20% (=PHB–co–HV 80/20) were implanted submucoperiostally on the palate of Beagle dogs. After 2, 4, 8 and 12 weeks *in situ*, the structure of the films and the tissue reactions were studied histologically. In terms of mechanical properties and tissue response, the PHB–co–HV film is the most suitable for use on dogs.

1. Introduction

Individuals with a surgically closed cleft lip, alveolus and or palate differ from the non-cleft population in facial morphology. Apart from other factors, the surgical repair itself is an important disturbing factor for maxillary growth [1–3].

Closure of palatal defects in humans is often achieved by the so-called von Langenbeck technique. With this technique, palatal closure is achieved by moving mucoperiosteal flaps to the midline of the palate, leaving two areas of denuded bone adjacent to the dentition. Reduction of the surface area of these wounds proceeds by migration of keratinocytes and fibroblasts as well as by wound contraction. Later on formation of scar tissue takes place which might influence subsequent maxillary growth or might affect the development of the dentition.

In order to study the iatrogenic effects of surgical palatal repair on growth and development, several animal experiments have been conducted in which soft tissue palatal repair was performed in a non-cleft Beagle dog model [4–7]. The results of the experiments simulating the von Langenbeck technique led to the conclusion that the scar tissue which developed on the areas of denuded bone became attached to the underlying bone by Sharpey's fibres. This phenomenon might be responsible for the disturbance of dento-alveolar development [6, 7]. Follow-up studies attempted to prevent the attachment of such scar tissue to the bone by submucoperiosteal insertion of porous poly-(L-lactic) acid (=PLLA) films (M_V = 220 kg/mol). Although it appeared that premature degradation of the films took place *in vivo*, the results were promising as no attachment of scar tissue to the bone was found in the vicinity of remnants of the films [8, 9].

The rate of degradation of biodegradable materials in specific *in vivo* circumstances is hard to predict as many interdependent material characteristics play a role, such as chemical structure, molecular mass, presence of residual compounds, crystallinity and physicochemical factors. Other factors of influence are sterilization, storage, interactions with tissues, and functional demands [10]. Valid data about the degradation rate can only be obtained if the test conditions mimic the real situation as closely as possible [11].

Disturbances in maxillary arch dimensions after palatal surgery in Beagle dogs become apparent during the transition of teeth [6]. Therefore, the mucoperiosteum and the bone have to remain separated until the transition of teeth has been completed. Surgery in Beagle dogs is performed when the deciduous dentition is completed at approximately 12 weeks of age. The transition is completed at about 24 weeks of age. Hence, the films have to remain intact for at least 12 weeks. The aim of this study is to evaluate the biodegradation of 5 different films after submucoperiosteal implantation on the palate of Beagle dogs.

2. Materials and methods

2.1. Materials

Films were made of five different materials: poly-(L-lactic) acid (=PLLA), high molecular weight poly-(L-lactic) acid (=HMW-PLLA), poly-(DL-latic) acid (=PDLLA), poly (ε-caprolactone) (=PCL) and a copolymer of poly hydroxybutyrate 80%-hydroxyvalerate 20% (=PHB–co–HV 80/20).

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PLLA and PDLLA were obtained from the Department of Chemical Technology, University of Twente, the Netherlands; HMW-PLLA was purchased from Purac Biochem, Gorinchem, the Netherlands; PCL from Interox Chemicals, Warrington, UK and PHB–co–HV was obtained from Marlborough Biopolymers, Cleveland, UK.

The viscosity average molecular weights (M_V) were determined by viscometry and calculated by applying the Mark–Houwink relationship [12]. For specifications of the materials given by the manufacturers see Table I.

All films were prepared at the Department of Chemical Technology, University of Twente, the Netherlands. The materials were dissolved as 5% w/w in chloroform (Merck, Darmstadt, Germany). After filtration they were cast on a glass plate in a thickness of 70–80 μm. After evaporation in a nitrogen gas atmosphere for 72 h, all films were vacuum dried at 80 °C for 48 h. The films were cut into samples 4 × 8 mm.

TABLE I Specifications of the films

Material	M_V[a]	T_m[b]	T_g[c]	Structure
PLLA	240	180	60	crystalline
HMW-PLLA	800	180	60	crystalline
PDLLA	460	–	55	amorphous
PCL	55	65	– 60	crystalline
PHB–co–HV	265	145	0	crystalline

[a] M_V = viscosity average molecular weight (kg/mol)
[b] T_m = melting temperature (°C)
[c] T_g = glasstransition temperatue (°C)

2.2. Implantation

In each of two young adult Beagle dogs films of five different materials were implanted at two points of time. The films were left for 2, 4, 8 or 12 weeks *in situ*.

After premedication with 0.5 ml Thalamonal® (fentanyl, 0.05 mg/ml + droperidol 2.5 mg/ml; Janssen Pharmaceutica, Beerse, Belgium) and 0.5 ml Atropine (atropine sulphate 0.5 mg/ml; Pharmachemie, Haarlem, the Netherlands) the animals were placed under general anaesthesia with 30 mg/kg Narcovet® (sodium pentobarbital 60 mg/ml; Apharmo, Arnhem, the Netherlands). The oral mucosa and the dentition were cleaned with chlorhexidine digluconate 1% in water. In addition 5 ml Xylocaine® (lidocaine hydrochloride 0.4 mg/ml + adrenaline 0.0125 mg/ml; Astra Chemicals, Rijswijk, the Netherlands) was injected into the palatal mucoperiosteum to avoid excessive bleeding during surgery.

To simulate the clinical situation where the films are in direct contact with the oral environment, a biopsy punch of diameter 4 mm (Stiefel Laboratorium, Offenbach am Main, Germany) was used to create five circular soft tissue defects between the rugae at one side of the palate at one-third of its width. Then an incision of approximately 8 mm was made 2 mm from the midline between the rugae. The mucoperiosteum was tunnelled from the incisions to the punched defects. In each pocket a film piece measuring 8 × 4 mm was inserted after disinfection by immersion in 1% chlorhexidine digluconate in water for 1 min followed by rinsing in sterile saline for 0.5 h. The part of the films underneath the defect was left uncovered. Suturing of the incisions was not necessary. The procedure was repeated at the other side of the palate for another series. A schematic drawing of the palate of a dog with films *in situ* is given in Fig. 1.

After surgery, the animals were medicated with 1 ml of Albipen® 15% (ampicillin anhydrate 150 mg/ml; Mycofarm, de Bilt, the Netherlands) and a maintenance dose of 1 ml Albipen® LA (ampicillin anhydrate 100 mg/ml; Mycofarm, de Bilt, the Netherlands) on

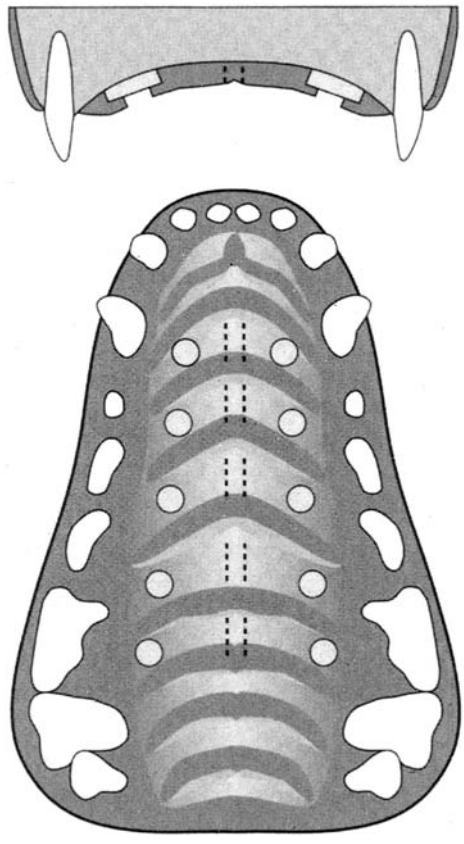

Figure 1 Schematic drawing of the palate of a dog, showing the soft tissue defects. The dotted lines represent the incisions. The transversal cross-section is showing the films *in situ*.

the second and fourth day postoperatively. The dogs received a normal diet after surgery.

At the end of the experimental period the animals were killed. After premedication with Thalamonal® they were placed under general anaesthesia using 30 mg/kg Narcovet® after which 0.5 ml/kg Thromboliquine® (heparin 5000 i.u./ml; Organon, Boxtel, the Netherlands) was administered. After some minutes a lethal dose of Narcovet® was injected intravenously. The vascular system was perfused with physiologic saline, followed by 4% neutral formaldehyde as a fixative. After perfusion, the maxillae were dissected and immersed in 4% neutral formaldehyde for another 2 weeks. They were then sawn into smaller blocks containing the specimens, which were decalcified in 20% formic acid and 5% sodium citrate, dehydrated, and embedded in Paraplast® (Monoject Scientific, Athy, Ireland). Serial sections of 7 μm were prepared

and stained with haematoxylin and eosin for histological examination.

Apart from routine histological parameters, a semi-quantitative classification was used for the description of the thickness of the capsule surrounding the films. A capsule consisting of 1–10 cell layers was called thin, 11–20 cell layers medium, and a capsule consisting of 21 or more cell layers was called thick.

3. Results

3.1. PLLA

After 2 weeks, the film could not be found in any of the sections; it had been apparently displaced. After 4 weeks the film was intact and was surrounded by a cellular capsule of medium thickness. The cellular component consisted mainly of macrophages, lymphocytes and plasma cells. The connective tissue in the vicinity of the film was hyperaemic. The palatal bone was lamellar. Neither bone resorption nor deposition was found. The film was displaced medially to some extent. After 8 weeks the film was still unimpaired. A fibrous capsule of medium thickness surrounded the film. The bone showed the same aspects as after 2 weeks. This film was also displaced medially. After 12 weeks the film was fractured into two fragments, each surrounded by a capsule of medium thickness. Close to the fracture, the capsule was interrupted by a focal infiltrate containing lymphocytes and plasma cells (Fig. 2). Some bone deposition was found in the vicinity of the film.

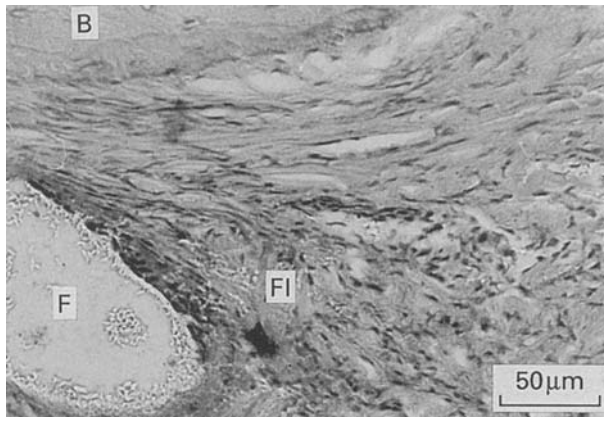

Figure 2 After 12 weeks the PLLA film was fractured and showed a focal infiltrate close to the fracture. F = film, B = bone, FI = focal infiltrate. H and E staining.

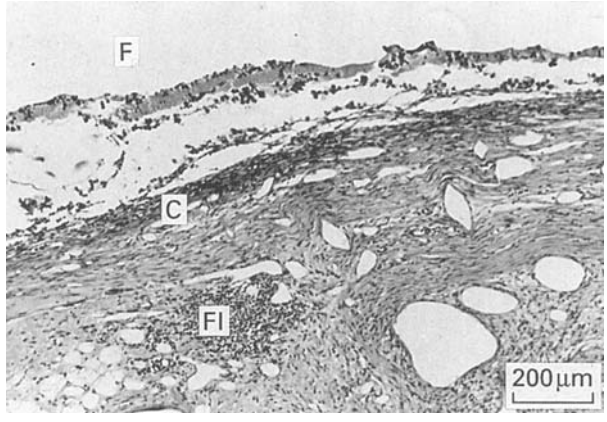

Figure 3 After 2 weeks the mucoperiosteum surrounding the HMW-PLLA film was hyperaemic. F = film, C = capsule, FI = focal infiltrate. H and E staining.

3.2. HMW-PLLA

After 2 weeks the film was slightly bent but fracture lines could not be found. The medium-sized capsule was partly cellular and partly fibrous. On the oral side of the film a focal infiltrate was located containing lymphocytes, plasma cells, macrophages and multinucleated giant cells which were also found in the cellular part of the capsule. The mucoperiosteum close to the film was hyperaemic (Fig. 3). Resorption of the lamellar bone was found underneath the film. After 4 weeks the film was surrounded by a thin fibrous capsule. No infiltrate was present. Some bone resorption was found. The 8-week film was slightly bent and showed some fracture lines. It was surrounded by a fibrous capsule of medium thickness. There was no infiltrate, and the bone showed local resorption. The 12-week film was fractured into two parts and showed a few focal infiltrates containing lymphocytes and plasma cells.

3.3. PDLLA

After 2 weeks the film was surrounded by a thin cellular capsule containing lymphocytes, plasma cells and macrophages. The film was not fractured and no infiltrates were found. The bone was lamellar and bone deposition might have been slightly reduced. After 4 weeks the non-fractured PDLLA film showed some cell ingrowth at the side of the film that faced the bone. On the same side the thin cellular capsule contained lymphocytes, plasma cells and macrophages. On the oral side of the film the capsule had changed into a fibrous one. Some bone resorption was found. There was no hyperaemia. After 8 weeks only a part of the film was present in the section. It was surrounded by a partly thin, partly medium cellular capsule containing lymphocytes and plasma cells. The film was unimpaired and showed more cell ingrowth at the bone side compared with the 4-week film. The deposition of bone was decreased. After 12 weeks the film showed a fracture line and was surrounded by a thin cellular capsule containing lymphocytes and plasma cells. The cellular capsule was surrounded by a thick fibrous capsule. A focal infiltrate was found close to the fracture line, containing lymphocytes and plasma cells. The cell ingrowth, also at the side facing the bone, was most marked in the 12-week PDLLA film (Fig. 4). The bone deposition was reduced.

3.4. PCL

After 2 weeks the film was folded without fracturing or fracture lines. The cellular capsule of medium thickness contained lymphocytes, plasma cells and macrophages. An infiltrate was located in between the folding, consisting of the same cells as the capsule. The

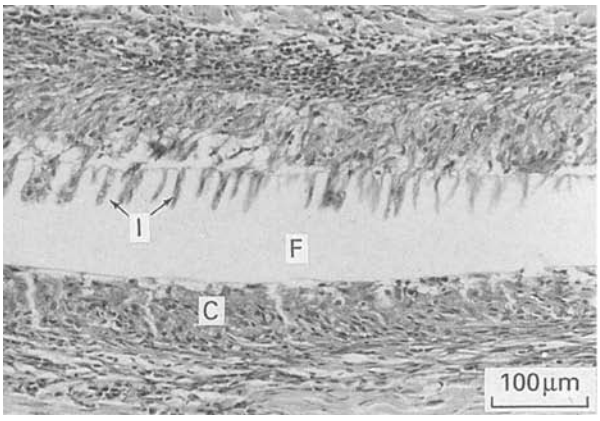

Figure 4 This 12-week specimen of the PDLLA film showed cell ingrowth on the side facing the bone. F = film, C = capsule, I = cell ingrowth. H and E staining.

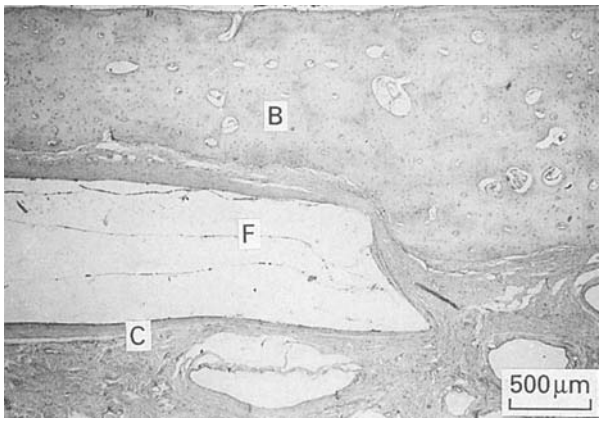

Figure 5 The folded PCL film was surrounded by a thin fibrous capsule after 12 weeks. Severe bone resorption could be noticed. F = film, C = capsule, B = bone. H and E staining.

tissue surrounding the film was hyperaemic, and moderate bone resorption was found. The bone was lamellar. After 4 weeks the film was also folded. The capsule was thin and severe bone resorption was found. The other aspects were the same as after 2 weeks of implantation. After 8 weeks the film was also folded. The fibrous capsule was of medium thickness. Severe bone resorption was found but no infiltrates were encountered. After 12 weeks the folded film was surrounded by a thin fibrous capsule. Severe bone resorption was still found (Fig. 5).

3.5. PHB–co–HV

After 2 weeks the film was deformed into an S-shape with some fracture lines in the curves (Fig. 6). Severe resorption of lamellar bone was found at the side where the film was close to the bone. A cellular capsule of medium thickness containing lymphocytes, plasma cells and macrophages surrounded the film. The implantation area was hyperaemic. An infiltrate could not be found. After 4 weeks the end of the film was bent, showing some fracture lines. It was surrounded by a thin fibrous capsule, and bone deposition was found. The films were unimpaired after 8 and 12 weeks. Both were surrounded by a fibrous capsule of medium thickness. There were neither infiltrates nor

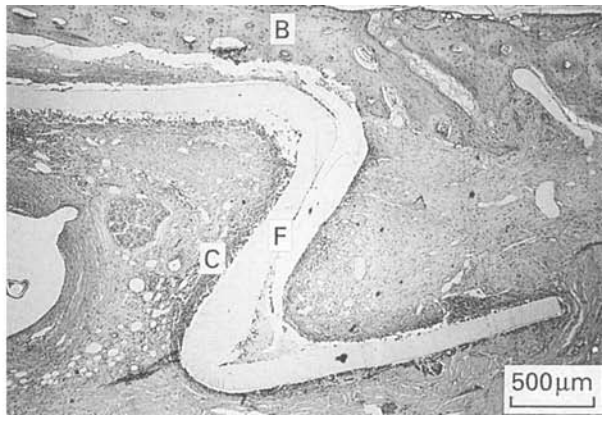

Figure 6 After 2 weeks the PHB–co–HV 80/20 was deformed in an S-shape. Severe bone resorption was found on the side where the film was close to the bone. F = film, C = capsule, B = bone. H and E staining.

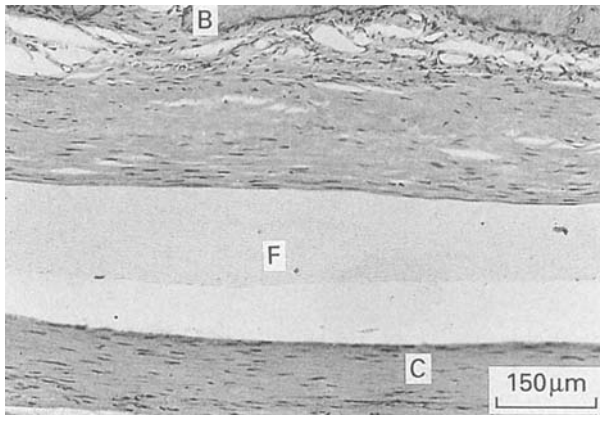

Figure 7 After 12 weeks the unimpaired PHB–co–HV 80/20 film was surrounded with soft tissue without signs of inflammation. F = film, C = capsule, B = bone. H and E staining.

hyperaemic regions. There was no osteoclastic activity (Fig. 7).

4. Discussion

The aim of this study was to investigate the *in vivo* behaviour of different biodegradable films after submucoperiosteal implantation on the palate of the Beagle dog. In the histological evaluation attention was paid to the localization and structure of the films; to the size, structure and components of the capsule; to the localization, size and components of infiltrates and to bone deposition and resorption.

The localization of the films was normal. They were found where expected, except for the PLLA films which had an unexplained tendency to displace medially; this also has been described by In de Braekt *et al.* [9]. Probably due to this tendency, the 2-week PLLA film was absent in the specimen.

Fracture was only observed in films consisting of PLLA and HMW-PLLA after 12 weeks. Both films fractured into two parts. This does not conform to the outcome of several other studies, where earlier fracture is reported. In de Braekt *et al.* [8,9] reported fracture of porous PLLA films (M_v = 220 kg/mol, thickness 110 µm, porosity range 0.5 µm to 1.0 µm)

into several small parts starting after the first week of implantation on the palate of Beagle dogs. Galgut *et al.* [13] reported fracture of PLLA membranes (M_V = 50 kg/mol and M_V = 200 kg/mol, thickness 20 μm, 40 μm and 70 μm) and of membranes made of a copolymer of PHB–co–HV 80/20 within 2 weeks after placement transcutaneously in the dorsum of rats. Beumer [14] also reported fragmentation starting 2 weeks postoperatively after implantations of bilayers existing of dense top layers of Polyactive® (HC Implants, Leiden, the Netherlands), a poly(ethylene oxide) – poly(butylene terephthalate) (=PEO/PBT) copolymer, with variable weight ratios of soft (PEO) and hard (PBT) segments and macroporous underlayers of the same material or of poly-L-lactide, M_W (weight average molecular weight) = 104 kD (Purac, Gorinchem, the Netherlands), porosity 100–200 μm.

In the present study, fracture of the PLLA and the HMW-PLLA films was only found after 12 weeks, which might indicate that non-porous material is more resistant to fracture than porous material, probably due to better mechanical properties and a smaller surface area available for (bio)chemical reactions. However, Li *et al.* [15–17] reported enhanced degradation of non-porous PLLA compared to porous material. They observed that the inner mass of PLLA degraded first, forming a semi-permeable layer on the outside. This layer prevents release of large parts of oligomers, which are thought to be responsible for inducing an autocatalytic process. This phenomenon was confirmed by Lam *et al.* [18] for PLLA films (M_V = 50 kg/mol, thickness 400 μm) of different porosity after subcutaneous implantation in rats. Due to the small thickness of the films used in our study (<100 μm), accumulation of oligomers and hence the autocatalytic process might not play a role. Higher load or load over larger surface area might also be reasons for a more rapid strength loss and earlier fracture of biodegradable films [19].

Deformation was found, for example, in the 8-week HMW-PLLA film and the 2-week PHB–co–HV film. This is probably due to the insertion procedure [13] and might be prevented by a surgical procedure in which the films are applied without stress. The folding seen in the PCL films might be the result of internal stress of the material and could passibly be prevented by changing the manufacturing procedures.

The capsule was generally of medium thickness and changed in time from a cellular capsule containing lymphocytes, plasma cells and macrophages to a more fibrous one. The PDLLA films were an exception showing cellular capsule at each point of time. The PDLLA films showed cell ingrowth increasing in time on the side facing towards the bone in the 4, 8 and 12 week specimens. The fact that cell ingrowth took place only at one side of the film might be explained by the casting procedure by which an irregular surface could have been formed. In all three specimens cell ingrowth was facing the bone, but this seems to be a coincidence.

Small focal infiltrates containing lymphocytes and plasma cells seemed to be related to fractures, fracture lines, or were located between the folded PCL films. Fragments of the polymers might probably induce cellular infiltration.

Resorption of the palatal bone was exclusively found in the vicinity of films showing deformation, i.e. the PCL films and to a lesser extent the HMW-PLLA films. This indicates that increase of pressure in the soft tissues caused by deformation of the films is a more important factor leading to bone resorption than chemical stimuli by degradation products.

The biodegradation of PLLA, HMW-PLLA, PDLLA and PCL is clearly demonstrated in many studies. In the literature there is some doubt about the biodegradation of PHB–co–HV. Miller and Williams [11] concluded that PHB and its PHV copolymers only degrade *in vivo* (subcutaneous implantation in rats) after subjection to 10.0 Mrad of gamma radiation. Others [20] concluded that the copolymer does degrade *in vitro*, although very slowly, thus PHB and its PHV copolymers do seem to be biodegradable material.

5. Conclusions

Compared with the other films, the PHB–co–HV 80/20 film is the easiest one to manipulate and adapt during surgery. The material induces very little tissue response after 8 and 12 weeks. Thus, with respect to the material characteristics and the tissue response, the PHB–co–HV films are the most suitable for our purpose. The other materials might also be usable after some modifications, as it is well known that degradation characteristics are dependent on the way the films are prepared. Although an increasing amount of literature is available on the degradation of PHB and its copolymers, supplementary research has to be carried out into the degradation rate of PHB–co–HV for this specific application.

It has to be emphasized that the results of this study cannot be translated directly to the human situation because of the specific *in vivo* degradation characteristics. When separation of mucoperiosteum and bone has been proven to lead to a more favourable dento-alveolar development in the dog model, further research has to be done to obtain suitable films for use in human cleft palate surgery.

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