Biodegradable materials are materials that degrade (decompose) in a biological environment. In the medical field they have been used for nearly 40 years in sutures and osteosynthesis materials. As a result of their degradability these devices do not have to be removed from the body after they have performed their function.

Nowadays, biodegradable materials are also applied in regenerative medicine. In this application, scaffolds made of biodegradable materials are used to engineer tissue outside the body or to guide regeneration of damaged tissue inside the body.

Most biodegradable materials are polymers. Amorphous copolymers of lactic acid and other monomers with an equivalent structure have proven to be most biocompatible, both initially and during degradation. The application of these copolymers is restricted, mainly as a result of their limited elastic and mechanical properties. These polyesters can be reinforced by connecting the polyester chains with degradable urethane segments, which results in biodegradable polyurethanes.

Until recently biodegradable polyurethanes were produced using aromatic diisocyanates. The degradation of these polyurethanes leads to the release of potentially carcinogenic components. The polyurethanes that were investigated in this thesis were synthesized from an aliphatic diisocyanate. The material consists of repeating units of hard urethane segments and amorphous soft segments based on a copolyester of DL-lactide and ε-caprolactone.

The urethane segments have a uniform length of five urethane moieties and are synthesized from 1,4-butanediisocyanate and butanediol. During degradation of the urethane segments butanediamine is formed. Butanediamine is normally present in the human body and can be excreted with the urine.

Before a new biodegradable material can be used safely and effectively in medical applications several aspects of biodegradation and biocompatibility have to be investigated. These include biocompatibility of the non-degraded material, biocompatibility during degradation and finally total resorption of the material. Depending on the application of the material other aspects have to be tested as well, such as the degradation at a specific anatomical location **(Chapter 1)**.

In this thesis highly porous foams of the polyurethane based on 1,4-butanediisocyanate were investigated. Because of the good mechanical and elastic properties the foam is a serious candidate for use in tissue regeneration applications. The performance of the foam in a biological environment had not been extensively investigated before the start of this research project. Therefore, several *in vivo* and *in vitro* biocompatibility and degradation experiments were performed to investigate the possibility of using the foams in medical devices for tissue regeneration.

In all biocompatibility and degradation studies (Chapter 2-5) the polyurethane foams were compared to high molecular weight copolymer foams made of DL-lactide and ε-caprolactone. This is a polyester of similar components as the soft segments of the polyurethane. The biocompatibility and biodegradation of this reference material has been extensively investigated.

The polyurethane foams were also tested in a specific application, that is the closure of oroantral communications. An oroantral communication is an open connection between the oral cavity and the maxillary sinus. This defect is mostly caused by extraction of (pre)molars from the maxilla. As a rule oroantral communications are closed surgically.

the defect with the biodegradable polyurethane foam. It is assumed that the good elastic properties of the polyurethane keep the foam in place while the oral and maxillary sinus mucosa are healing.

Before research into the application started several aspects of the short-term biocompatibility of the polyurethane foam were evaluated **(Chapter 2).**

The *in vitro* cytotoxicity of the non-degraded foam was established first. Fibroblasts were cultured and were either directly exposed to the polyurethane or exposed to extracts of the material. After incubation periods of 24 to 72 hours the cytotoxicity was evaluated by counting the cells, by measuring their metabolic activity, and by a morphological evaluation. In these tests the polyurethane caused no abnormal growth behaviour, nor morphological changes or inhibition in metabolic activity.

To establish the *in vivo* biocompatibility of the non-degraded polyurethane small foam discs were implanted subcutaneously on the back of rats for one, four and twelve weeks. The tissue response to the material was histologically evaluated. These studies did not show any toxic tissue response to the polyurethane. The foams provided an inviting matrix for connective tissue ingrowth, accompanied by vascular ingrowth. From these short-term experiments it was concluded that the non-degraded polyurethane shows a good biocompatibility, both *in vitro* and *in vivo.*

Most problems regarding the biocompatibility of biodegradable polymers occur after longer periods of implantation. Nowadays, nearly all investigations into the long-term safety of biodegradable materials are still being performed with animal experiments.

In **Chapter 3** a new method is proposed to test the long-term *in vitro* cytotoxicity of biodegradable materials by testing the toxicity of their degradation products. The study was performed to develop a method that saves time, laboratory animals and research funds. The biodegradable polyurethane foam and the copolymer control material were subjected to this new test method.

Accumulated degradation products of the materials were collected by degrading the foams in distilled water at 60°C up to 52 weeks. Cell culture medium was prepared from powder medium using this water. In several tests, comparable to the methods of Chapter 2, the cytotoxicity of this medium was established.

The first signs of cytotoxicity were observed after 3 to 5 weeks degradation. This accounted for both materials and re-established the good short-term biocompatibility of the polyurethane and the reference material.

The polyurethane showed more toxicity towards the end stages of degradation in comparison with the copolymer control. This is probably related to the accumulation of degradation products of the urethane segments. It was speculated that the mechanisms for metabolism and excretion of these degradation products, which are available in the *in vivo* situation, prevent toxic reactions *in vivo*.

In **Chapter 4** the degradation of the highly porous polyurethane and copolymer control foams was evaluated *in vitro*. The foams were degraded in a Sörensen buffer solution (pH 7.4) at 37°C and 60°C for time intervals up to 3 years. The remaining material was collected on microporous filters. Dimensions of the foams, intrinsic viscosity, mass loss, thermal properties and composition of the remaining material were evaluated.

The polyurethane foams kept their dimensions for 20 weeks at 37°C. The copolymer

foams collapsed after 3 weeks and lost 60% of their volume. PU mass loss reached a maximum of 80% at both 37°C and 60°C. CP mass loss reached 99.9% at 60°C, and 92% at 37°C after 3 years.

Analysis of the 37°C and 60°C results showed that the degradation processes are initially the same, but eventually degradation products with different thermal properties are being formed. ^{1}H-NMR studies showed that the hard urethane segments of the PU do not degrade *in vitro*.

It was concluded that the polyurethane material has favourable characteristics for a scaffold material. Total resorption, however, can still be best investigated with *in vivo* studies. These were described in **Chapter 5.**

In a long-term subcutaneous implantation study polyurethane foam discs were implanted in rats and rabbits for intervals up to 3 years. The copolymer foam of DL-lactide and ε-caprolactone served again as a control. The foams and surrounding tissues were evaluated with light and transmission electron microscopy, with respect to tissue response during degradation and, ultimately, the resorption of the material. At later stages of degradation lymph nodes draining the implantation sites were evaluated for the presence of remnants of the polyurethane.

During degradation, temporary elevated numbers of macrophages and giant cells were observed in both the polyurethane and the control samples. Electron microscopy showed that the macrophages contained pieces of the polyurethane. The size of the intracellular polyurethane particles diminished and the cells containing these remnants gradually disappeared after periods from 1 to 3 years. After 3 years an occasional isolated macrophage with biomaterial remnants could be traced in both the polyurethane and the copolymer explants. A few macrophages with biomaterial remnants were temporary observed in the lymph nodes in the periods between 39 weeks and 1.5 years.

It was concluded that the PU foam is biocompatible during degradation. After 3 years PU samples had been resorbed almost completely. Based on the results, local intracellular degradation, followed by removal of the low molecular degradation products through the blood stream seems the most likely route of elimination of the material. The results indicate that the PU foam can be safely used as a biodegradable implant. They also indicate that the *in vitro* results of Chapter 4 are only partly representative for the *in vivo* situation and therefore total resorption has to be investigated in long-term *in vivo* studies.

Before a medical device can be applied in humans, the application should be tested in animal experiments. For the investigations into the closure of oroantral communications (OACs) animal models were not readily available. In **Chapter 6** a pilot study was performed to investigate whether the Göttingen minipig is a suitable animal model for creating and closing OACs. The application of the polyurethane foam in the defects was tested as well. In three adult minipigs the left and right second maxillary molars were extracted. An OAC was created by drilling from the extraction socket to the maxillary sinus. The left side was closed by a standard surgical buccal flap procedure, which is comparable to the surgical closure performed in humans. The right defects were treated by applying the biodegradable polyurethane foam. After 2 weeks, 1 month and 3 months the pigs were sacrificed for histological evaluation.

Postmortal and histological examination showed that an OAC was created only in one

of six cases. In the remaining cases the infraorbital canal was perforated instead of the floor of the maxillary sinus. It was concluded Göttingen minipig is not a suitable animal model, due to the location of the infraorbital nerve. As a result the closure of the OACs with the biodegradable polyurethane foam could not be evaluated. The first observations of the polyurethane implanted in the maxilla showed a mild tissue response to the material. There were also some indications that the oral mucosa could not grow rapidly across the foam.

In **Chapter 7** another pilot study is described. In that study the suitability of rabbits to serve as an animal model for the closure of OACs was investigated. Six rabbits were used, and an OAC was created on both sides of the maxilla. Three rabbits were used to evaluate the animal model by applying a surgical treatment on one side and by leaving the other defects untreated. The results were evaluated after 1, 2 and 4 weeks of healing. Histological evaluation showed that OACs had been created in the rabbits. The surgically treated defects healed without complications. The untreated defects showed complicated and delayed healing. It was therefore concluded that the rabbit is a suitable animal model.

In the three other rabbits OACs were closed with polyurethane foam. Based on the results of chapter 6 some modifications in the composition of the soft segment of the polyurethane were introduced. The polyurethane with the 'standard' composition of the soft segments (DL-lactide and ε-caprolactone) was used to close half of the defects. The other defects were closed with a polyurethane to which 23% polyethylene glycol was added to a glycolide/ε-caprolactone soft segment to make the material more hydrophilic.

Although closure of the OAC was achieved, the foam with 23% polyethylene glycol degraded too fast for our purpose. Just as in the minipigs, the standard polyurethane did not guide the regeneration of the oral mucosa very well.

In **Chapter 8** a PU with an intermediate composition, with 5% polyethylene glycol added to the DL-lactide/ε-caprolactone soft segment, was used to close OACs in rabbits. Nine rabbits were used and an OAC was created on both sides of the maxilla. In this experiment standard surgical closure was compared to closure with the polyurethane foam. Healing of the defects was evaluated by clinical and histological examination, after periods from 1, 2, 4, and 10 weeks.

The surgically treated defects healed uneventfully. The oral side of the foam treated defects had clinically closed after 4 weeks. Some of the foams occupied a relatively large part of the small maxillary sinus. Nevertheless, the maxillary sinus mucosa gradually grew across the polyurethane foams. Regeneration of maxillary bone was observed in both the foam-treated defects and the surgical control. It was concluded that the polyurethane foam with 5% polyethyleneglycol provides adequate closure of an OAC in the rabbit animal model to allow healing of the defect.

In **Chapter 9** the results of the former chapters are compared and discussed.

Because of the limitations of *in vitro* experiments (Chapter 2,3 and 4) the *in vivo* experiments (Chapter 2 and 5) can still be considered as 'the golden standard'. Based on the *in vivo* experiments it was concluded that the polyurethane shows a satisfactory biocompatibility, both initially and during degradation. After three years the foams are at the end stage of degradation. The observations with the electron microscope indicate that the resorption has not stopped and it is very likely that the material will ultimately be totally resorbed.

The investigation into the closure of oroantral communications has been performed in a relatively small number of animals. Nevertheless, this treatment method can now be tested in long-term animal experiments with a polyurethane with a degradation rate and hydrophilicity that is modified according to the needs of the application. The very satisfactory results of the biocompatibility and biodegradation experiments even make it possible to introduce the material in human studies in the near future.