In vivo and *in vitro* degradation of poly[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL], and the implications for the use in nerve reconstruction

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Abstract: Nerve guides can be used for the reconstruction of peripheral nerve defects. After serving their function, nerve guides should degrade. p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] degrades completely within 1 year without the formation of a slow degrading crystalline fraction. Although the tensile strength (TS) of a p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guide is negligible after 2 months, nerve regeneration across a 1-cm gap in the sciatic nerve of the rat is faster and qualitatively better than after reconstruction using autologous nerve grafts. During degradation p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] swells, especially during the first 3 months. This can have a negative influence on the regenerating nerve. p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guides could only be used in the clinical situation in case of short nerve gaps (several mm) in small nerves (for instance digital nerves). Refinements will be needed to successfully reconstruct longer nerve gaps (several cm). © 2000 John Wiley & Sons, Inc. J Biomed Mater Res, 51, 575–585, 2000.

Key words: p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL]; *in vivo* and *in vitro* degradation; nerve guide, nerve regeneration; effective surface; ε-CL and LLA-crystals

INTRODUCTION

Biomaterials are defined as "non-viable materials used in a medical device, intended to interact with biological systems."[1] Application of biomaterials for the replacement, improvement, or reconstruction of diseased or damaged organs/tissues is of great interest. The concept behind the use of biodegradable materials is that no foreign body material will be left in the host when a biomaterial has performed its function.[2] One example is the use of nerve guides for the reconstruction of peripheral nerve gaps.[3,4]

The general concept behind the use of nerve guides is that regenerating nerve fibers are allowed to grow toward the distal nerve stump, while neuroma formation and ingrowth of fibrous tissue into the nerve gap is prevented. Furthermore, inside the nerve guide tropic and trophic factors, produced by the distal nerve stump, can accumulate.[5] Trophic factors are necessary for the survival and growth of the damaged axons, and tropic factors are necessary for the direction of the growth of the regenerating axons.[6] The direction of the growth of the regenerating axons is not only caused by a mechanical effect (the wall of the nerve guide), but also by a chemical effect (the gradient of trophic factors).

In the past, several materials, either from biological origin[7–9] or synthetically fabricated (both biodurable[10,11] and biodegradable[12,13]), have been tried for this purpose with more or less success. After the nerve fibers have bridged the gap between the proximal and distal nerve stumps, the nerve guide becomes useless, and may even have a negative influence on the regenerated nerves.[14,15] Merle et al.[14] reported successful nerve regeneration after reconstruction of peripheral nerves in three patients, with a silicone nerve guide. After 2 years, the patients began complaining about secondary nerve impairment and irritation at the implantation site. A second operation was needed to remove the biodurable silicone rubber nerve guides. Because the removal of a biodurable nerve guide might

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damage the regenerated nerves, nerve guides should degrade immediately after serving its function. Fine-tuning of the degradation of nerve guides, however, is extremely important for the final result of the nerve reconstruction.

Besides the fact that the degradation rate of the nerve guide should be in accordance with the axonal growth rates, nerve guides have to fulfill several other requirements. The biomaterial should be biocompatible, which means that it should be noncytotoxic, noncarcinogenic, nonimmunogenic, nonmutagenic, and cause no irritation or allergic response, either local or systemic. Furthermore, a nerve guide should be flexible, (semi)permeable and easy to apply in microsurgery. Preferably, a nerve guide should also be transparent. Besides the transparency, which allows accurate observation of the nerve stumps when telescoping them into the nerve guides, (semi)permeability of the wall of the nerve guides has a positive effect on the nerve regeneration.[16,17]

Robinson et al.[18] tested a two-ply polyurethane nerve guide, since good results were obtained with two-ply polyurethane blood vessels. This nerve guide, however, had several disadvantages: it was relatively hard and brittle, and more serious: the degradation products of the polyurethane were cytotoxic. Furthermore, the polyurethane did not degrade completely.

Later, a semicrystalline copolymer of L-lactide and ε-caprolactone (50:50) was prepared and tested.[19] This material proved to be noncytotoxic.[20] Furthermore, nerve regeneration after reconstruction with this type of nerve guide was good. However, after 2 years, fragments of the nerve guide material were still present around the regenerated nerve, causing a chronic foreign body reaction with scar tissue formation.[21] Rozema et al.[22] described similar problems with bone plates made of crystalline poly(L-lactic acid) (PLLA), which were used for the fixation of zygoma fractures. After 3 years, all patients showed a severe, well-defined swelling, strictly limited to the implantation site. In the case of seven patients it was decided to surgically remove the swelling. At light microscopic examination, the removed tissue was characterized by a foreign body reaction without signs of inflammation. At transmission electron microscopic level large amounts of highly crystalline PLLA particles were observed. It was concluded that the degradation of the PLLA osteosynthesis material was very slow and that the total degradation time was much longer than 3 years, as could also be estimated on the basis of *in vitro* and *in vivo* degradation studies.[23]

To overcome this problem, D-lactide was added to obtain a biomaterial with a lower crystallinity than PLLA and consequently a higher degradation rate.[24] The first results with this poly(96L/4D-lactide) are promising.[24] As the degradation of the nerve guides, constructed of a semicrystalline copolymer of L-lactide and ε-caprolactone was also too slow, it was decided to add D-lactide to obtain a faster degrading nerve guide.

MATERIALS AND METHODS

Polymer

The resulting biodegradable copolymer consists of 50% lactide (LA) and 50% ε-caprolactone (ε-CL). The lactide component contains 85% L-lactide (LLA) and 15% D-lactide (DLA).

First the monomers were purified using standard procedures.[19] Ring-opening polymerization was performed in a silanized polymerization tube after addition of stannous-dioctanoate, which was used as a catalyst. After homogenizing the components, polymerization took place at a temperature of 130°C during 22 days. The polymer was purified by precipitating it in a mixture of 6:4 (v/v) hexane/aceton.

The polymer has a weight averaged molecular weight (M_w) of 1.1×10^6 kg/kmol, a polydispersity index of 2.5, and a glass transition temperature (T_g) of −12°C. The polymer is completely amorphous, which means that it does not contain a crystalline fraction. The biomaterial is transparent and rubber-like.

Two types of p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] devices were constructed: 1. bars for subcutaneous *in vivo* evaluation of the degradation, and 2. nerve guides for *in vitro* evaluation of the degradation.

Preparation of the bars

A solution of 3 wt % in chloroform was made. A film of polymer was made by solvent casting in a petri dish. The film was washed in a mixture of 8:1 (v/v) ethanol/water for 15 min to clear the copolymer from monomers and solvent. After air-drying, the next layer could be cast over the film. This procedure was repeated several times and resulted in a polymer disc with a thickness of 3 mm. From this disc bars were cut with the following dimensions: 3 × 3 × 15 mm. These bars were EtO-sterilized before subcutaneous *in vivo* implantation in the rat.

Preparation of the nerve guides

A solution of 3 wt % in chloroform was made. A glass mandrel was dipped in this solution with a speed of 1.6 mm/s, stayed in the solution for 10 s and was pulled out of the solution with the same speed. The glass mandrel was placed horizontally, and a rotation procedure was started immediately to overcome variations in wall thickness. After evaporation of the chloroform, the nerve guide was washed in a mixture of 8:1 (v/v) ethanol/water for 15 min. After air-drying the second layer could be dip-coated on top of the

first. The nerve guides were manually removed from the glass mandrel, and had the following dimensions: internal diameter 1.5 mm, wall thickness 0.30 mm, length 14 mm. The nerve guides were EtO-sterilized and then tested in an *in vitro* degradation study. It should be mentioned that at the start of the *in vitro* degradation study, the M_w was 6.5×10^5 kg/kmol.

Light-microscopical analysis of the degrading copolymer

p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] specimens with surrounding tissue were harvested monthly up to 1 year. The specimens were fixed in glutaraldehyde 2% (0.1*M* phosphate-buffered) for 2 h at room temperature. Subsequently, the specimens were washed and dehydrated in a graded ethanol series. Embedding was performed in glycol methacrylate (Technovit 7100, Kulzer GmbH, Wehrheim/Ts, Germany). Sections (1.0 μm) were cut and stained with alkaline fuchsin and toluidine blue. The specimens were evaluated for light microscopical signs of degradation.

Polymer–chemical analysis of the degrading copolymer

The degrading copolymer was tested for M_w, changes in composition and the possible presence of crystals. Before analyzation, the degraded copolymer specimens were dried in a vacuum oven at 40°C for 3 days. Molecular weights were determined using gel permeation chromatography in combination with light scattering, using chloroform or tetrahydrofuran as solvents. The composition of the copolymer was determined by ^{1}H-nuclear magnetic resonance using a Varian 200 MHz spectrometer. Deuterated chloroform was used as a solvent. A Perkin Elmer-7 Differential Scanning Calorimeter, Perkin-Elmer Europe bv, Nienwerkerk ald Yssel, The Netherlands, was used to detect the eventual presence of poly(L-lactide), as well as, poly(ε-caprolactone) crystals.

The swelling behavior of the degrading biomaterial *in vitro* was evaluated by measuring the dry and wet mass of the sample and to compare this with the initial mass. *In vivo*, standardized specimens (bars) were implanted: 3 × 3 × 15 mm. On the explanted specimens tissue remnants remain. Therefore, it was chosen to measure the size and not the mass. From these measurements the volume was calculated and compared with the initial volume of the bar (135 mm^3).

The TS of the degrading nerve guides *in vitro* was examined at room temperature on a Zwick 1445 (Zwick GmbH, Ulm, Germany) operating at a crosshead speed of 12 mm/min.

RESULTS

Light-microscopical analysis of the degrading copolymer

Immediately after implantation, a fibrous capsule was formed around the subcutaneously implanted bars. After 3 months the first cracks appeared at the surface. Cellular extensions protruded in these cracks. After 4 months the outer layer started to fragment and cells started to migrate between the fragments (Fig. 1). At this period the volume of biomaterial had decreased, but fragments of biomaterial could not be observed on the outside of the fibrous capsule or in the fibrous capsule itself. At 5 months, however, fragments could be observed in the capsule itself and 6 months after implantation fragments of biomaterial, surrounded by foreign body giant cells could be observed on the outside of the capsule. At 6 months, the first signs of diffusion of protein and ingrowth of cells into the p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] were observed. Furthermore, at this time macrophages with phagocytosed biomaterial could be observed. The amount of diffused proteins, as well as the number of ingrowing cells increased with time. After 11 months some small fragments of biomaterial could be observed, surrounded by connective tissue. After 12 months only connective tissue remained at the site of implantation.

Polymer–chemical analysis of the degrading copolymer

Molecular weight decrease of bars and nerve guides

The M_w of the subcutaneously implanted bars was nearly constant during the first 3 months. However, at 3 months, the viscosity chromatogram showed both a large peak, resembling 1.1×10^6 kg/kmol (i.e., the initial M_w), and a small peak, resembling a M_w of 2.0×10^5 kg/kmol. These smaller molecules probably originate from the surface of the bars, which starts to degrade earlier/faster than the center of the bar. This idea is supported by the fact that first the outer part of the bars starts to fragment (Fig. 1). After 3 months the M_w decreased sharply from 1.1×10^6 kg/kmol to 2.9×10^3 kg/kmol after 6 months (Fig. 2). From the molecular weight distribution after 8 months, a small fraction with a molecular weight between 200 and 500 kg/kmol (e.g., di- tri- and tetra-mers) could be observed.

The M_w of nerve guides *in vitro* was also evaluated. These nerve guides had an initial M_w of 6.5×10^5 kg/kmol and a polydispersity index of 1.5. The M_w decreased steadily from 6.5×10^5 kg/kmol to 2.8×10^3 kg/kmol after 6 months.[25]

Swelling of nerve guides and bars during degradation

From *in vitro* degradation studies with nerve guides it is obvious that the degrading biomaterial is able to take up increasing amounts of water with time [Fig.

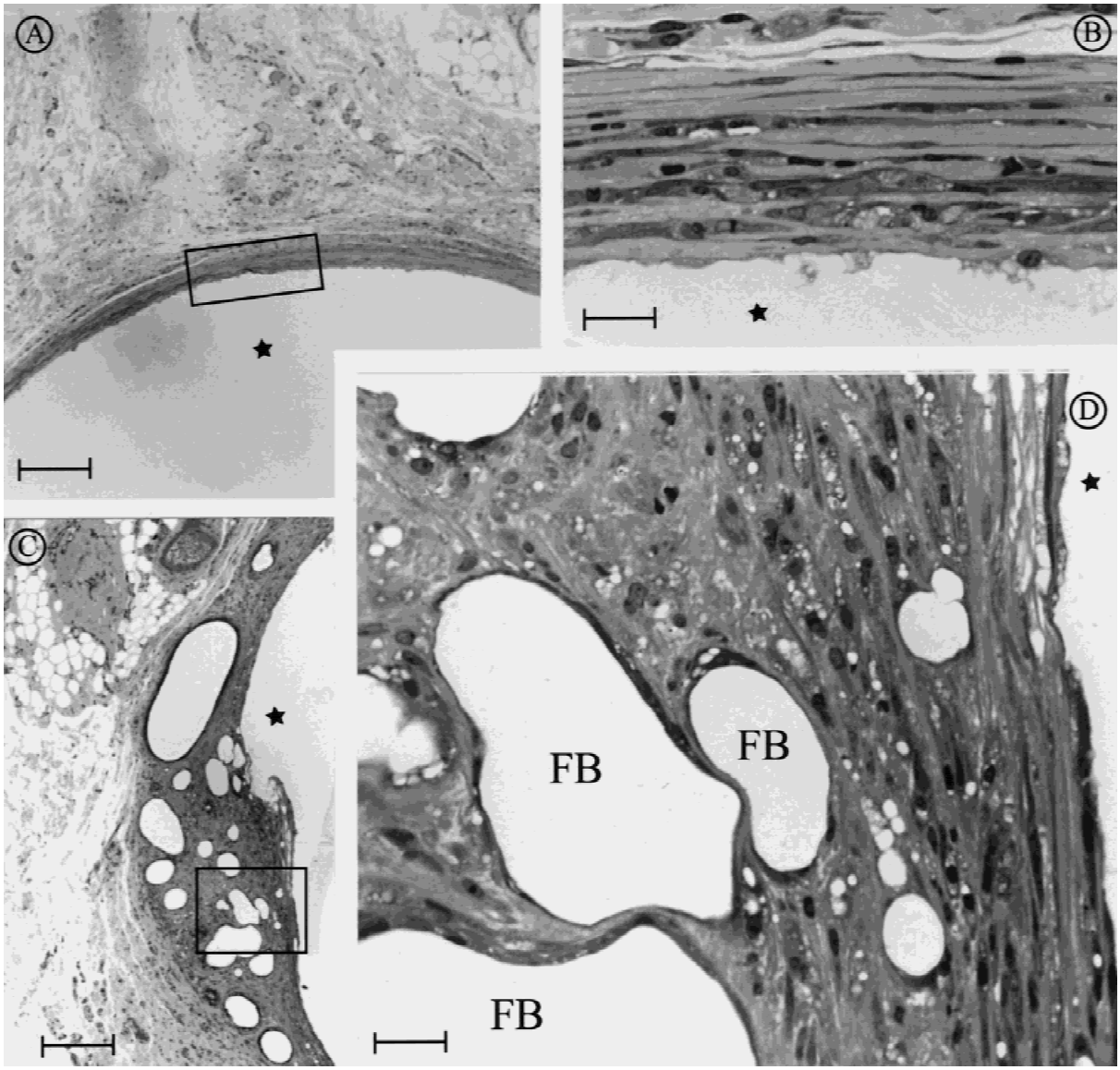

Figure 1. Light micrographs of degrading p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] bars after 3 (A) and (B) and 4 months (C) and (D). (B) and (D) are details from (A) and (C), respectively. Note that after 4 months, the outer layer of the bars is fragmented, and that cells migrated between these fragments. * Represents the biomaterial. FB represents the fragments of biomaterial. The bars in (A) and (C) represent 170 μm and the bars in (B) and (D) represent 17 μm.

3(a)], while the amount of dry mass is decreasing [Fig. 3(b)]. This was also observed in the subcutaneous degradation study of the bars. The volumina of the bars increased enormously between 1 and 3 months to approximately 300% of the originally implanted volume (e.g., there was 200% water uptake) [Fig. 3(c)]. After 3 months, the volume of the subcutaneous implants decreased sharply. After 8 months of implantation, less than 5% of the originally implanted volume remained. The ability of water uptake per amount of biomaterial increases to a greater extent after 3 months [Fig. 3(a)]. The mass loss of the dry weight of the biomaterial also increases after 3 months [Fig. 3(b)]. It is obvious that these changes occur at the same time as the decrease of the M_w of the biomaterial [Fig. (2)]. However, water uptake and mass loss in the *in vitro* tested nerve guides occur steadily, as is the case with the M_w decrease. In the case of the bars, a decrease in both volume and M_w occurred suddenly at 3 months. The differences in the swelling characteristics between the *in vitro* tested nerve guides and subcutaneously implanted bars are probably caused by: 1. the dimensions of the nerve guides and the bars, and 2. the study conditions—*in vitro* versus *in vivo* (including cellular activity).

Because of ingrowth of fibrous tissue, fragmentation of the biomaterial will occur earlier (when compared with *in vitro* degradation studies), resulting in a higher degradation rate. A good example of the influence of cellular activity on fragmentation is the fact that *in*

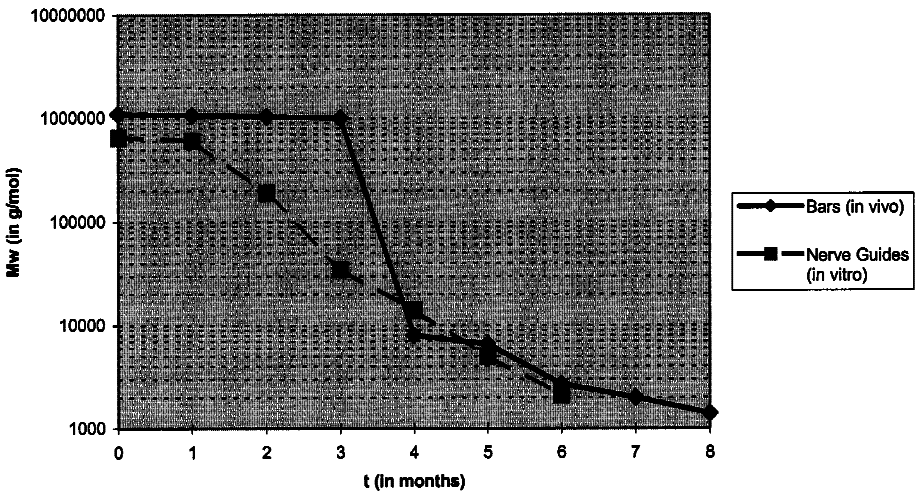

Figure 2. Graph showing the change in M_w during degradation of bars (*in vivo*) and nerve guides (*in vitro*). The M_w of the bars is nearly constant during the first 3 months. Thereafter, the M_w decreases sharply. After 8 months the M_w is 1.4×10^3 g/mol. At this time a small fraction with a molecular weight between 200 and 500 could be observed (i.e., dimers, trimers, and tetramers of lactide and ε-caprolactone). The decrease in M_w of the nerve guides starts immediately. After 6 months the M_w is 2.1×10^3 g/mol.

vitro nerve guides are still in one piece after 5 months, whereas *in situ* implanted nerve guides are totally fragmented after the same period[26] [Fig. (4)].

Recrystallization of p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] during degradation *in vitro* and *in vivo*

Before implantation, the p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] is completely amorphous, and the original T_g is −12°C. During degradation, melting peaks at 40° to 50°C could be observed, probably caused by recrystallization of ε-caprolactone–rich segments (oligomers) in the copolymer. In some cases, small melting peaks at 125°C could be observed, probably caused by recrystallization of L-lactide–rich degradation products. Furthermore, in some cases a second T_g could be observed between 25° to 40°C, which points to a lactide-rich phase. Besides ε-caprolactone–rich crystals, small lactide-rich crystals are formed. These lactide-rich are small and not very pure, since purified poly(L-lactide) has a melting point at 188°C instead of the measured 125°C. *In vitro*, the same results were obtained, although melting peaks from lactide-rich segments were observed less often than *in vivo*.

TS measurements

During degradation of nerve guides *in vitro*, the TS decreased from 2.5 MPa (at $t = 0$ weeks) to 0.1 MPa (at $t = 8$ weeks). After longer periods, TS measurements could not be performed. These results are in accordance with the decrease in M_w (Fig. 5). The TS as well as the M_w of the nerve guides start to decrease after a period of 3 to 4 weeks. After this period, both the M_w and TS decrease. When the M_w has become less than 200,000 kg/kmol, the TS becomes negligible.

DISCUSSION

Molecular weight decrease versus nerve regeneration

Nerve guides have to fulfill several requirements: guiding outgrowing nerve fibers toward the distal nerve stump while preventing neuroma formation and ingrowth of fibrous tissue. After serving this function, the nerve guide may disintegrate/degrade. The rate at which the nerve guide should degrade should be in accordance with the axonal growth rates, which are dependent on the type of nerve lesion, any concomitant lesion, site of injury, type of species, age of species or patients, etc.[35]

After reconstruction of a 1-cm gap in the sciatic nerve of the rat with a 12-mm p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guide, axon regeneration starts after a few days and is quite fast: the first nerve fibers have crossed the 1-cm gap inside the nerve guide within 3 weeks.[29] After this period, the number and diameter of the nerve fibers inside the nerve guide increase. After 10 weeks the regenerating nerve inside the nerve guide has almost matured.[29] After this period, the nerve guide is no longer necessary. The M_w decrease of nerve guides *in vitro* (Fig. 2) starts immediately. After 10 weeks (e.g., 2.5 months), the M_w has decreased from 6.5×10^5 kg/kmol to 1.0×10^5 kg/kmol. Although the M_w decrease starts immediately, good

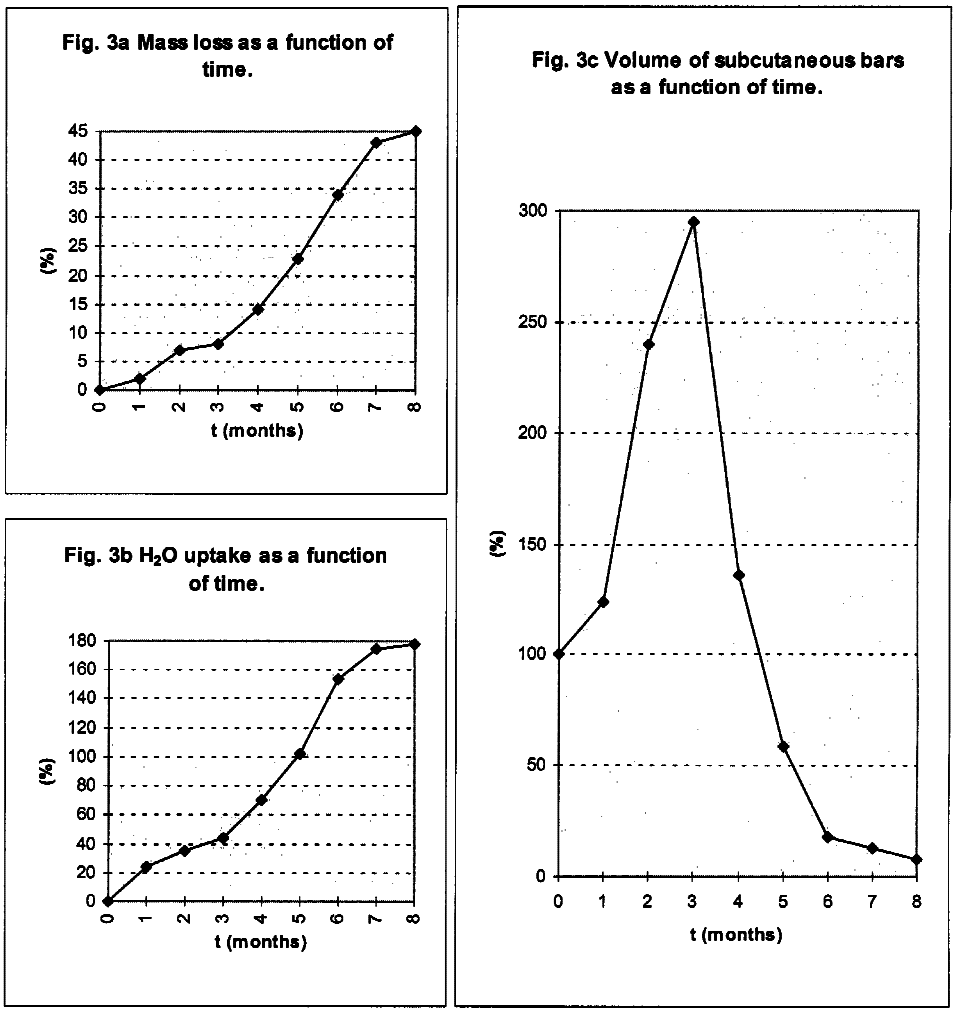

Figure 3. (a) Shows the percentage of mass loss of degrading nerve guides *in vitro* as a function of time. (b) Shows the percentage of water uptake of the degrading nerve guides *in vitro*. Note that after 3 months both the mass loss and water uptake increase. (c) Shows the volume of the subcutaneously implanted bars as a function of time. The volume indicated here is the sum of the volume of biomaterial and the volume of water taken up by the degrading biomaterial. Note that the volume of the degrading bars also sharply decreases after 3 months.

quality nerve regeneration can be assured in the case of a 1-cm gap in the sciatic nerve of the rat. Furthermore, after ≈10 weeks (e.g., when the nerve guide is no longer necessary) the M_w of the biomaterial decreases faster (Fig. 2), and probably does not affect the regenerated nerve. After 5 months the nerve guides are totally fragmented (Fig. 5),[26] and negative influences on the regenerated nerve are not observed.

In humans, however, nerve regeneration is not as fast as in rats (Fig. 6). First there is a latency period of 3 weeks after nerve transection, in which no signs of axon regeneration at the reconstruction site can be observed. After 3 weeks, the severed axons start to regenerate with a speed of ≈1 mm/day. Therefore, in the case of a 1-cm nerve gap in humans, the first regenerating axons entering the distal nerve stump can be expected between 4 and 5 weeks. Besides a slower growth of axons in humans, maturation will also occur more slowly. Because nerve fibers regenerate more slowly in humans, the degradation of p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guides might be too fast to assure full maturation of the regenerating nerve fibers in a 1-cm nerve gap. To evaluate whether a p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guide would function properly in the case of nerve reconstruction in a patient, it might be necessary to test pre-degraded nerve guides in an animal model, in order to mimic the latency period.[34]

Swelling of the degrading biomaterial versus nerve regeneration

Besides the M_w decrease of the biomaterial, swelling of the degrading material is very important with regard to peripheral nerve regeneration. The bulk/morphology of a piece of biomaterial is very important with regard to the degradation and the swelling.

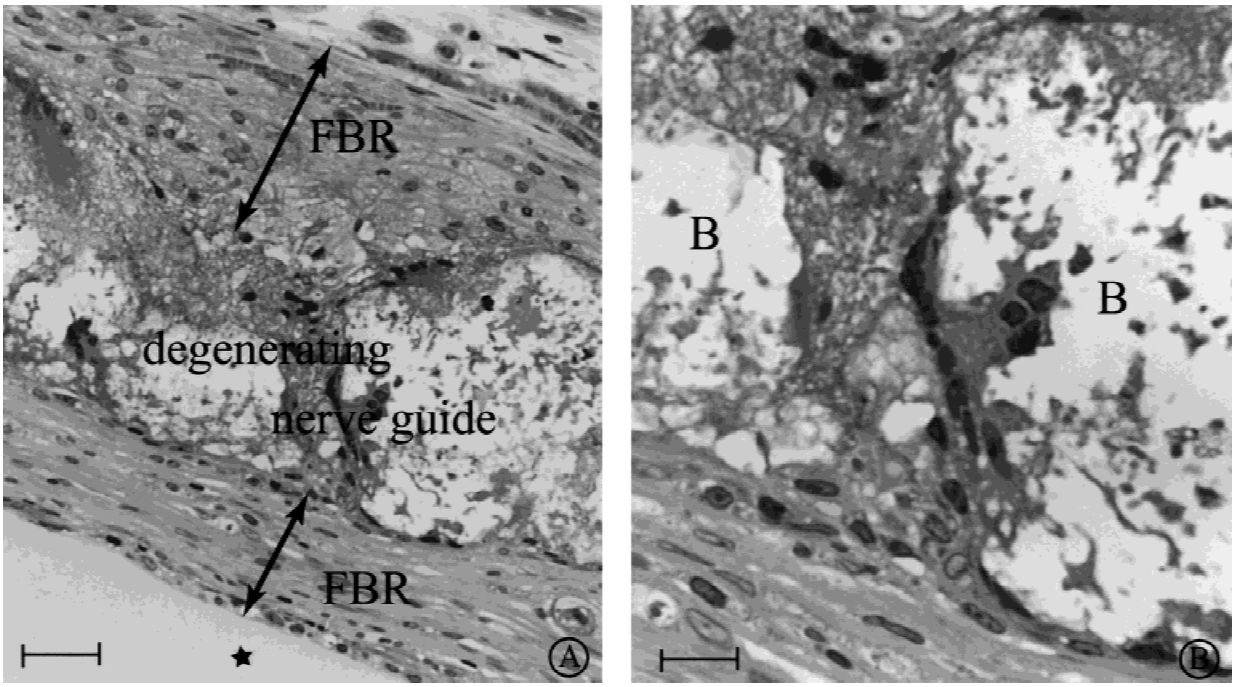

Figure 4. Light micrographs of a p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guide, 5 months after degradation. Note that the biomaterial (e.g., nerve guide wall) is totally fragmented. * Represents the luminal side of the nerve guide. FBR = foreign body reaction. (b) is a detail from (a). Note that cells migrate between the fragments of biomaterial. The bar in (a) represents 26 μm and the bar in (b) represents 10 μm.

Comparing the bars with the nerve guides, it can be concluded that the larger the bulk of biomaterial, the more swelling of the degrading biomaterial occurs. To compare different implants, we introduced the "effective surface,"[26] which is the ratio between the surface and the volume of an implant.

In Table I, the calculations of the effective surfaces of various implants are listed. It can be observed that the bars (evaluated in the subcutaneous degradation study) have the smallest effective surface, whereas the nerve guides (evaluated in the *in vitro* degradation study) have the largest effective surface. From the results described previously, and those listed in Table I, it can be concluded that the larger the effective surface, the less swelling of the degrading biomaterial.

This conclusion is supported by the results of a previous study[27] in which 4 p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guides with different dimensions were used for the reconstruction of 1-cm nerve gaps (i.e., *in situ* implantation). Nerve guide types III and IV had the lowest effective surfaces (more bulk; see also Table I) and showed a more pronounced swelling, starting 1 month after implantation. The swelling was so severe that by 2 months the lumina of these nerve guides were completely blocked and the regenerating nerves had to grow on the outside of the nerve guide. Nerve guide type II (with a higher effective surface) showed a less pronounced swelling, but still the nerve regeneration was not preferable. Nerve guide type I (with the highest effective surface) functioned best, causing no constriction of the regenerating nerve because of swelling of the degrading biomaterial.

Besides the effective surface of a biodegradable prosthesis, the internal structure of the prosthesis might influence the degradation rate. Both the bars and nerve guides were composed of multiple layers of polymer. The bars were made by solvent cast in a petri dish and the nerve guides by dip-coating. The bars, however, were composed of more layers then the nerve guides. This difference might cause the bars to degrade more slowly. To evaluate this more deeply, however, bars composed of various layers should be compared.

Study conditions (*in vitro* versus *in vivo*) might have an even greater impact. Cellular activity plays a role in

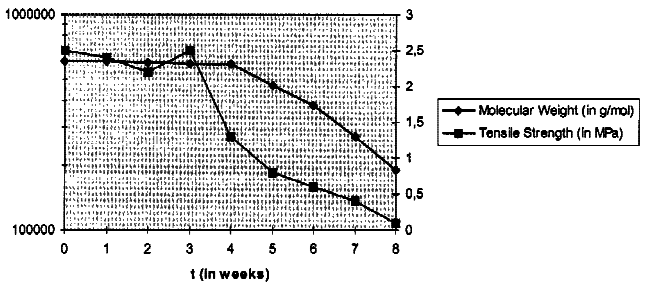

Figure 5. Graph showing the decrease of M_w and TS during *in vitro* degradation of nerve guides. Note that the M_w is nearly constant during the first 4 weeks. After this period the M_w decreases more sharply, whereas the TS starts to decrease sharply after 3 weeks.

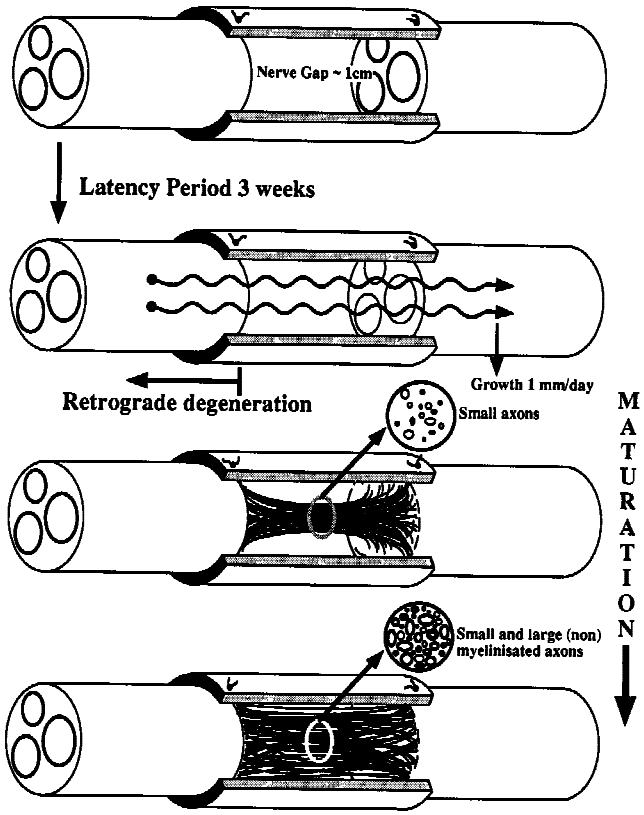

Figure 6. Scheme representing nerve regeneration through a p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guide after reconstruction of a 1-cm nerve gap in a patient. First there is a latency period of 3 weeks. Then axons start to regenerate through the last part of the proximal nerve stump, due to retrograde degeneration. The speed of the axon regeneration is approximately 1 mm/day. After 4 to 5 weeks, the first axons have entered the distal nerve stump. After this period, the nerve has to mature: increase in number and diameter of nerve fibers and myelinization.

the fragmentation of the biomaterial (Figs. 1 and 4). From Figure 1 it can be observed that the surface of the bar is fragmented after 4 months, whereas the center seems intact. *In vitro* cracks are formed in the biomaterial, but it is still in one piece after 4 months. Because of fragmentation, the effective surface becomes greater, thereby increasing the degradation rate. Furthermore, the fibrous tissue capsule that surrounds the implant might act as a barrier for oligomers. Because of the presence of more free end-groups more water is absorbed, in turn leading to a more pronounced swelling. Evaluation of *in vivo* and *in vitro* degradation of exactly the same implant size and form, as well as internal structure could elucidate the influence of cellular activity. Whether enzymes or oxygen radicals, produced by the surrounding tissue, play a role in the degradation of the biomaterial was not evaluated in this study.

With regard to the swelling, it was concluded that a p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guide with an internal diameter 1.5 times larger than the diameter of the severed nerve would function best.[27] When the internal diameter is smaller, it is more difficult to telescope the nerve stumps into the nerve guide, and if the internal diameter is too large, fibrous tissue can grow into the nerve guide, hampering the nerve regeneration. When the wall is too thick (smaller effective surface), swelling of the nerve guide during degradation can be so severe that the lumen is occluded.[27] However, if the wall is too thin, the nerve guide will collapse in an early phase of the degradation.[32,37]

In later experiments,[29,30] nerve guides with a high effective surface (e.g., the same as the nerve guides that were evaluated in the *in vitro* degradation study) were used with great success: nerve regeneration through these nerve guides is the fastest described thus far,[29] and even faster and qualitatively better than nerve regeneration through autologous nerve grafts[30] (which is the commonly used technique in the clinical situation). Furthermore, functional nerve recovery proved to be very good after nerve reconstruction using these nerve guides.[31]

Recrystallization versus nerve regeneration

During degradation a second T_g occurred between 25° and 40°C, pointing at a lactide-rich phase. In addition, ε-caprolactone and small lactide-rich crystals were formed. These crystals, however, were very impure and would probably not decrease the degradation rate of the biomaterial.

de Groot et al.[28] evaluated porous 50:50 copoly(L-lactide/ε-caprolactone) implants. They observed that during degradation an increasing melting endotherm appeared between 100° and 120°C, corresponding with crystallized L-lactide sequences, which are less susceptible to hydrolysis. In previous studies, we used the same material as a two-ply biodegradable nerve guide.[19–21] It was observed that after 18 months, a large number of biomaterial fragments lay around the regenerated nerve. Because of the presence of these slow degrading fragments, much fibrous scar tissue was formed, surrounding the nerve. Ducker and Hayes[15] and Merle et al.[14] have shown that fibrous scar tissue, surrounding a silicone-rubber nerve guide, could cause constriction of the regenerated nerve on the long term, thereby negatively influencing the nerve function.

In the case of p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL], small lactide and ε-caprolactone–rich crystallites are formed, but they are so impure that the degradation rate of the biomaterial will probably not be influenced. Furthermore, within 1 year the biomaterial has completely disappeared, without a chronic foreign body reaction with scar tissue formation.[26]

TABLE I
Calculation of the Effective Surface of the Nerve Guide Used in the *In Vitro* Study, the *In Situ* Implanted Nerve Guides (I to IV)[27] and the Subcutaneously Implanted Bars

Type of Implant	Internal		External		Volume (mm^3)	Surface (mm^2)	Effective Surface
	r	r^2	r	r^2			
Nerve Guide	.70	.49	1.00	1.00	16.02	110.02	6.87
Type I	.62	.38	.95	.90	16.49	101.63	6.16
Type II	.59	.34	1.02	1.03	21.58	105.15	4.87
Type III	.58	.33	1.21	1.46	35.59	119.28	3.35
Type IV	.56	.31	1.24	1.53	38.04	120.38	3.16
Bars	3 × 3 × 15 mm				135.00	198.00	1.47

The volume of the nerve guides was calculated as follows: $\pi l(r_{ext}{}^2 - r_{int}{}^2)$. The surface was calculated as follows: $2\pi l(r_{ext} + r_{int}) + 2\pi(r_{ext}{}^2 - r_{int}{}^2)$. The effective surface is the ratio of the surface and the volume.

TS versus nerve reconstruction and nerve regeneration

Because of hydrolysis of the p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL], crack formation occurs, resulting in loss of TS of the nerve guides. The speed at which the TS decreases can have a great impact on the quality of the nerve reconstruction as well as the quality of the peripheral nerve regeneration. First of all, the wall of the nerve guide should be strong enough in order to prevent the sutures from being torn out of the material, and losing continuity. On the other hand, the nerve guide should be flexible, in order to perform the implantation more easily. A p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guide with an internal diameter of 1.5 mm and a wall thickness of 0.3 mm, and an initial TS of 2.5 MPa has these requirements. A nerve guide with the same internal diameter, but with a wall thickness of 0.15 mm is strong enough to prevent tearing of the material due to suturing, but not strong enough to prevent collapse of the nerve guide due to pressure on the outside. Meek et al.[37] observed that 95% of these thin-walled nerve guides collapsed after implantation, resulting in a worse quality of nerve regeneration, in turn leading to a worse recovery of nerve function. When modified denatured muscle tissue[36] was used as a stent inside the nerve guide, collapse was prevented and both nerve regeneration and recovery of nerve function were better.[32] It can therefore be concluded that the initial strength of a nerve guide is primarily necessary to prevent collapse of the nerve guide. Furthermore it can be concluded that the strength, which is necessary to prevent tearing of the material due to suturing, is less than the strength to prevent collapse of the nerve guide.

As was previously described, nerve regeneration across a 1-cm gap in the sciatic nerve of the rat is fast; the regenerated nerve has a mature aspect after a period of 10 weeks.[29] After 8 weeks, however, the TS has decreased to 0.1 MPa, and cannot be measured thereafter. It can therefore be concluded that although the nerve guide has lost its strength completely, nerve regeneration has advanced so far that pressure on the outside does not influence the nerve regeneration anymore.

In the case of humans, nerve regeneration is slower, and the forces on the nerve guide will be greater. It might, therefore, be possible that a p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guide is not strong enough throughout the whole period which is necessary to cross the nerve gap completely. To prevent tearing of the nerve guide due to forces along the nerve guide, it might be necessary to immobilize joints using plaster.

Possible refinements of the p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guides with regard to the clinical situation

From the above it can be concluded that a nerve guide constructed of p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] can perfectly be used in the case of a 1-cm gap in the sciatic nerve of the rat,[27,29] and is even better than autologous nerve grafts.[30] The nerve guide degrades completely within 1 year,[26] without the formation of slow degrading crystals, which could cause a chronic foreign body reaction,[21] in turn leading to constriction of the regenerated nerve.[14,15] The degrading nerve guides lose their TS after approximately 2 months.[27] This period, however, is long enough to assure both the outgrowth and maturation of the nerve fibers through a 1-cm gap.[29]

Often in the clinical situation, however, longer nerve gaps will have to be reconstructed. Furthermore, peripheral nerve regeneration in humans is slower than in rats. Therefore, refinements of the p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] nerve guide will be necessary. Refinements can be obtained at different levels: 1. the polymer itself, 2. a different composition of the nerve guide wall, and 3. addition of growth factors, extracellular matrix molecules and/or Schwann cells inside the nerve guide.

p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] degrades fast, swells

during degradation, and loses its strength after 2 months. In the clinical situation it might be preferable to have a stronger biomaterial, which swells less during degradation, but which has all the other requirements: flexible, transparent, noncytotoxic,[27] no formation of slow degrading crystals,[26] and only a very mild foreign body reaction without the formation of fibrous scar tissue.[26] One way to obtain a material with these characteristics is to construct a network by forming crosslinks between the copolymeric molecules. In this manner, surface degradation will be more prominent than bulk degradation, and the biomaterial will degrade more slowly. Furthermore, because random chain scission cannot occur so easily anymore, less end groups will be present inside the material, and therefore less water will be absorbed, in turn leading to less swelling of the degrading biomaterial. In this manner it might be possible to successfully reconstruct longer nerve gaps. Although the swelling of a nerve guide constructed of a p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] network will probably be less, an internal diameter which is 1.5 times the diameter of the severed nerve will still be necessary since this is important with regard to the fibrin matrix formation inside the nerve guide, which functions as a primary scaffold for the nerve regeneration across the nerve gap.[40,41]

Another way to improve the nerve guide is to make the wall, or a part of the wall, porous. In this way, the swelling of the nerve guide during degradation will be limited. In previous studies[19–21] a two-ply nerve guide with a dense inner layer and a porous outer layer was tested. Fibrous tissue could grow into the porous outer layer, without hampering the nerve regeneration inside the nerve guide, functioning as a kind of pseudosynovial sheath[38,39] that assures further maturation of the reconstructed nerve, even when the biomaterial has lost its TS.

Last but not least, other forms of support of peripheral nerve regeneration across longer nerve gaps are possible, such as growth factors,[42,43] extracellular matrix molecules,[44,45] and Schwann cell seeding.[46,47] The addition of substances inside nerve guides, however, should be performed with great care: in a pilot study conducted by us (unpublished results), a 0.5% rat-collagen gel was added. First it was observed that regenerating nerve fibers did not grow through the gel, but between the collagen gel and the nerve guide wall. After a few weeks, a severe foreign body reaction to the collagen gel could be observed, with the formation of fibrous scar tissue, which hampered the nerve regeneration. Valentini et al.[45] also showed, that collagen- and laminin-containing gels (which positively influence axonal growth *in vitro*) impede peripheral nerve regeneration through nerve guides. From the study conducted by Rizvi et al.,[48] it became clear that longitudinally aligned collagen fibers supported nerve fiber regeneration as long as they stayed intact. As soon as they fell apart, the nerve regeneration was hampered. It can therefore be concluded that an additional factor inside a nerve guide should also guide the outgrowing nerve fibers toward the distal nerve stump (e.g., a gel does not contain a three-dimensional structure which guides outgrowing nerve fibers).

From the above it can be concluded that with some refinements p[$^{50}/_{50}$ ($^{85}/_{15}{}^{L}/_{D}$)LA/ε-CL] can be used in the construction of nerve guides, which might later be used in the clinical situation. Furthermore, a large number of additional factors might enhance peripheral nerve regeneration. A complete review regarding these factors, however, does not fit within the scope of this article.

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