

# Knitted polylactide 96/4 L/D structures and scaffolds for tissue engineering

## Shelf life, in vitro and in vivo studies

Ville Ellä,<sup>1,\*</sup> Tuija Annala,<sup>2</sup> Satu Länsman,<sup>3</sup> Manu Nurminen<sup>1</sup> and Minna Kellomäki<sup>1</sup>

<sup>1</sup>Department of Biomedical Engineering; Tampere University of Technology; Tampere, Finland; <sup>2</sup>Scaffdex Ltd.; Tampere, Finland;

<sup>3</sup>Department of Ophthalmology; Oulu University Hospital; Oulu, Finland

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This study covers the whole production cycle, from biodegradable polymer processing to an in vivo tissue engineered construct. Six different biodegradable polylactide 96/4 L/D single jersey knits were manufactured using either four or eight multifilament fiber batches. The properties of those were studied in vitro for 42 weeks and in 0- to 3-year shelf life studies. Three types (Ø 12, 15 and 19 mm) of cylindrical scaffolds were manufactured from the knit, and the properties of those were studied in vitro for 48 weeks. For the Ø 15 mm scaffold type, mechanical properties were also studied in a one-year in vivo experiment. The scaffolds were implanted in the rat subcutis. All the scaffolds were  $\gamma$ -irradiated prior to the studies. In vitro, all the knits lost 99% of their mechanical strength in 30 weeks. In the three-year follow up of shelf life properties, there was no decrease in the mechanical properties due to the storage time and only a 12% decrease in molecular weight. The in vitro and in vivo scaffolds lost their mechanical properties after 1 week. In the case of the in vivo samples, the mechanical properties were restored again, stepwise, by the presence of growing/maturing tissue between weeks 3 and 12. Faster degradation was observed with in vitro scaffolds compared to in vivo scaffolds during the one-year follow up.

### Introduction

Poly lactides (PLA), purified and medical-grade forms of polymers have been used for tissue repair and reconstruction applications for decades. Due to their good processability, suitable properties and biodegradability as well as non-toxic degradation products, it is still an interesting polymer family for biomedical and TE purposes.<sup>1</sup> An important factor when considering commercial products is that several products made of PLAs exist that already have FDA approval or an equivalent permit.

Monofilaments and multifilament fibers have been produced from PLA polymers with varying properties, depending on the processing parameters.<sup>2-4</sup> PLA fibers can be used as such, e.g., as sutures to close soft tissue wounds,<sup>5</sup> but they are also versatile preforms from a manufacturing perspective. Methods familiar from the textile industry can be used to produce various kinds of textile structures using continuous fibers as well as stapled ones, but fibers as well as textile structures can be used as elements in composites too.

The applicable techniques include, for example, non-woven methods<sup>6-8</sup> and knitting<sup>9-14</sup> to produce porous structures for guided tissue ingrowth and tissue engineering purposes. Though textiles can be used as such, they can also be used as preforms to

prepare three-dimensional structures, like scaffolds, e.g., for bone reconstruction<sup>15</sup> and for small joint reconstructions.<sup>16-19</sup>

It is well known that for tissue engineering scaffolds, specific pore size is needed to enable cell ingrowth, =tissue formation and maturation,<sup>20</sup> and interconnective pores are essential for constructing a viable 3D tissue engineered construct.<sup>20,21</sup> It is also easy to modify the parameters of the textile production and monitor the influences of the production to the finished product,<sup>22</sup> and thus, by varying the parameters, several products with very different properties can result.

In this paper, we covered the process from raw material to TE product. We studied the mechanical and molecular properties of manufactured fibers made of poly(L/D)lactide 96/4, tubular single jersey knits made of fibers and cylindrical scaffolds prepared from the knits. We studied the differences of the manufacturing parameters of the knits, and we assessed their long-term stability in hydrolysis in buffer solution at 37°C. For the knits, we also studied the long-term storage stability, so called shelf life, for up to 3 years. We were also in position to study both in vitro and in vivo degradation for up to 1 year for the cylindrical scaffolds and to compare their mechanical behavior under compression. The importance and relationship between the tissue ingrowth and the mechanical properties were assessed.

\*Correspondence to: Ville Ellä; Email: ville.ella@tut.fi

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**Table 1.** Knitting parameters for six knit types

Sample	Filament Count	Barrel Size	Needles in the Barrel	Loop Size	Course/cm	Wales/cm	Loop Length (mm)
4F	4						
8F	8						
8F-9Ns	8	½"	9	Small	8.1	6.6	6
8F-9Nb	8	½"	9	Big	4.1	4.2	11
4F-9Nb	4	½"	9	Big	4.1	4.2	11
4F-9Ns	4	½"	9	Small	8.1	6.6	6
4F-19Ns	4	½"	19	Small	8.1	6.6	6
8F-19Ns	8	½"	19	Small	8.1	6.6	6

## Results

**Initial properties of the fibers and knits.** Measured single fiber diameters for 4-ply fibers were 78–82  $\mu\text{m}$  and for the 8-ply fibers, 68–70  $\mu\text{m}$ . The initial mechanical properties of the non-irradiated 4- and 8-ply multifilament fibers after extrusion and hot drawing were measured (Table 3). The higher number of filaments had a statistically significant increase in tensile load, but the cross-sectional area-related properties (strength and modulus) were statistically significantly higher for the 4-ply yarn bundle. The ultimate break load per yarn was an average 57% higher with the 4-ply fiber and 75% higher with the 8-ply fiber when compared to the yarns in the knits. Due to the structure type of the knits, there was a drastic (> 99.3%) decrease in modulus when comparing the knits and the fibers. For the strain properties, there was an average increase of 17% in the 4-filament 9-needle group and a 38% increase in the 4-filament 19-needle group. For the 8-filament 9-needle group, there was no change in average values, but for the 19-needle knit, there was a 17% increase in strain properties.

The loop size statistically significantly affected the maximum load of 4F-knit, but differences between small and big loop size were not statistically significant in 8F-knit. Similar behavior was noticed for the modulus values, where the modulus is statistically significantly increased by the bigger loop size—68% for 8F-knit and 63% for the 4F-knit. The loop size against strain showed an increase in 4F-knit when the loop size was bigger, but this difference was not statistically significant. However, decrease of strain in the 8F-knit was statistically significant with smaller loop size. Increase of number of needles from 9 to 19 also had a statistically significant effect on the properties of the knits.

**In vitro results of the knits.** When the load values were plotted against the incubation time (Fig. 3), the influence of the number of yarns on the absolute load properties is clearly seen, giving the 19 needle and 8F knits higher load values. When the strength values were plotted against the incubation time (Fig. 4), we noticed that the batches with 4-ply knits will start to lose their strength after 9 weeks and 8-ply knits after 15 weeks. At the beginning, the 4-ply knit is stronger until week-12, after which it will lose strength more rapidly. The difference between 12 and 15 weeks is statistically significant.

The average starting (Table 1) strain levels (32–44%) of the knits decreased along the load values and dropped down to ~10%

between the weeks 9 and 21. The deepest drop can be noticed with 4F-19Ns, which dropped from 41% to 12.6% between weeks 9 and 15. All mechanical properties of the tested knits were statistically significantly decreased after 12 weeks in vitro.

The initial  $M_w$  of the raw material was 280 kDa, and after the extrusion and  $\gamma$ -irradiation, the  $M_w$  dropped statistically significantly down to 32 kDa for both of the fiber batches. There was a constant decrease in molecular weights and in viscosity during the incubation (Fig. 5 and 6), which achieved statistically significant levels in 9 weeks. A steady increase in polydispersity can be seen for both 4F and 8F between the weeks 30 and 42. However, the only statistically significant change in polydispersity was measured in 42 weeks for 4F.

**Results of the shelf life tests.** In the shelf life tests, all the specimens retained their mechanical properties during the studied 3-year period (Table 4). No statistically significant differences were indicated by Student t-test ( $p > 0.05$ ) in comparison with mechanical properties of 0 weeks and 3 years. All the mechanical values showed a similar behavior. There was no difference in standard deviations for 0- or 3-year samples.

The combination of storage time and storing conditions slightly influenced the molecular properties of the stored  $\gamma$ -irradiated knits. We noticed that there was a statistically significant decrease when comparing the 0-week knits  $M_w \sim 32$  kDa and  $M_n \sim 18$  kDa to 3-year ones  $M_w \sim 28$  kDa and  $M_n \sim 15$  kDa (Fig. 5). There was an average 12% drop in the molecular weights during the 3-year study. The 2.5 year samples still retained similar molecular properties to those in the beginning. This time point is clearly seen on the PD curves (Fig. 6), which all show a definitive increase after 2.5 years. PD at 2.5 years was ~1.55, whereas at the 3-year point it was ~1.85. Otherwise steady measurements were disturbed at the 1.5 year study point for all the studied groups. This is, very likely the result of an error with the GPC equipment, or the 1.5-year samples were somehow corrupted in the analysis. Unfortunately, it was not possible to re-perform measurements afterwards.

**In vitro results of the cylindrical scaffolds.** The average porosity of the scaffolds (measured dry from un-incubated samples) is from ~81 to 87% (Table 2). The pore size distribution was calculated using the CT data. For a 15 mm scaffold, it was calculated that there were 42% < 100  $\mu\text{m}$  pores, 35% 100–200  $\mu\text{m}$  pores, 19% 200–300  $\mu\text{m}$  pores and 4% of >300  $\mu\text{m}$  pores.

For all the scaffold sizes ( $\phi$  12, 15 and 19 mm), the compression tests (Fig. 7) showed similar stiffness behavior. At week 1,

**Table 2.** Manufacturing details for 12, 15 and 19 mm scaffolds

Scaffold	Average Scaffold Diameter (mm)	Average Scaffold Height (mm)	Length of Knit (mm)	Average Weight (g)	Average Porosity %
12	12.3 ± 0.2	3.3 ± 0.2	120	0.135 ± 0.004	86.8 ± 2.6
15	14.8 ± 0.2	3.6 ± 0.3	200	0.22 ± 0.04	83.7 ± 1.0
19	19.3 ± 0.3	4.7 ± 0.3	350	0.36 ± 0.04	80.9 ± 8.7

**Table 3.** Preliminary mechanical properties for the yarns and the knits

YARNS	Load at Max Load (N)	Stress at Max Load (MPa)	% Strain at Max Load (%)	Modulus (GPa)	Yield Stress (MPa)	% Strain at Yield (%)
4-ply (n = 10)*	11.9 ± 0.8	581.2 ± 44.1	31.9 ± 2.2	8.0 ± 0.8	191.0 ± 9.9	2.8 ± 0.1
8-ply (n = 5)*	16.6 ± 0.7	561.6 ± 19.5	35.4 ± 3.6	7.1 ± 0.2	148.1 ± 2.8	2.6 ± 0.1
KNITS	Load at Max Load (N)	Load/ <sub>1/2</sub> loop (N)	% Strain at Max Load (%)	Modulus (MPa)		
8F-9Ns (n = 8)	91.7 ± 4.3	5.1 ± 0.2	39.2 ± 2.3	23.9 ± 1.5		
8F-9Nb (n = 8)	96.6 ± 10.1	5.4 ± 0.6	33.4 ± 4.3	40.3 ± 4.3		
8F-19Ns (n = 8)	186.8 ± 20.6	4.9 ± 0.5	41.6 ± 5.1	16.5 ± 0.9		
4F-9Nb (n = 8)	89.3 ± 8.2	5.0 ± 0.5	39.1 ± 7.7	30.0 ± 4.3		
4F-9Ns (n = 8)	67.8 ± 3.1	3.8 ± 0.2	35.9 ± 2.5	18.4 ± 1.0		
4F-19Ns (n = 8)	127.2 ± 18.6	3.3 ± 0.5	44.1 ± 4.7	11.4 ± 0.6		

\*Non  $\gamma$ -irradiated.

the 19 mm scaffold had lower compression values compared to the other two scaffolds. For all the scaffolds, after one week in vitro, there was a major reduction in compression stiffness, and the specimens lost their initial rigidity for the rest of the incubation period. The similar stiffness behavior referred to here was the minimal capability of the scaffolds retaining the compression load after 1-week incubation during a 1.5 mm compression.

GPC results show (Figs. 8 and 9) that the degradation rate of the PLA96 fibers in scaffolds in vitro is similar to the degradation behavior of the fibers 4F and 8F. After 42 weeks, the measured molecular weights were the same,  $M_w \sim 8$  kDa and  $M_n \sim 4$  kDa. As for the PD, we can see a definite rise starting from the week 20 PD  $\sim 1.6$  and rising up to 2.3 at week 42. There is a higher PD rise in the scaffold series compared to the plain fibers (4F and 8F). The molecular properties between the surface and the core of in vitro scaffolds showed no statistically significant differences during the in vitro incubation.

The weight loss of the three scaffold sizes was noticed to begin around the week 28. At week 48, there was a 10% mass loss observed for all the scaffold sizes. When the 15 mm sample weight loss data is plotted against the GPC results (Fig. 10), we can observe that the weight loss starts when the molar mass is less than 20 kDa, and it increases more rapidly when the molecular weight is less than 10 kDa.

**In vivo results of the cylindrical scaffolds.** We can see from the compression test curves (Fig. 7A–D) that the scaffold loses its stiffness in vivo before the week-2 test point. After 12 weeks, we can see a rise in stiffness, and it is further increased until week 48.

The degradation rate of the PLA96 fibers in scaffold format in vivo is statistically significantly different after 6 weeks when compared with the degradation rate in vitro. Implanted scaffolds

after of 52 weeks had  $M_w$  18 kDa and  $M_n$  12 kDa, whereas it was only 8 kDa after 48 weeks in vitro. However, both of the degradation profiles follow the same trend for the first 4 weeks, after which we can see a delay in the in vivo degradation that eventually leads to a slower degradation compared to the in vitro degradation. The viscosity is also statistically significantly different compared to in vitro samples. There is a clear lag in the viscosity drop in vivo, and in the case of PD, we cannot notice any statistically significant changes.

The 52-week specimens were visually examined (Fig. 11), and we can see that the scaffold is still present, visible, maintaining its form and tightly packed in dense connective tissue.

## Discussion

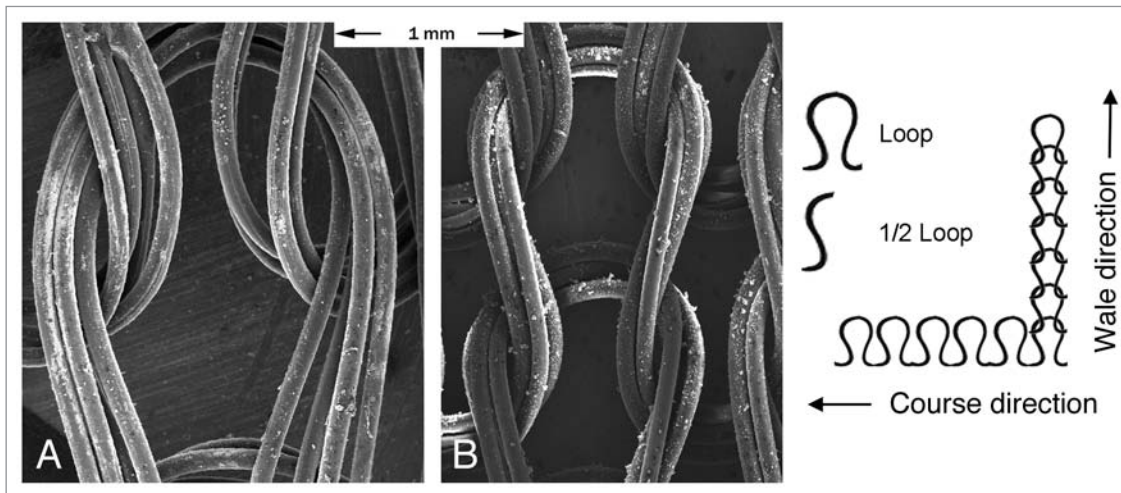
The tensile strength of the yarn in the knit is lower than that of the yarn tested prior the knitting. This is due to damage done to the fibers during the knitting as well as due to the non-unidirectional forces inflicted on the yarn due to the loops. For our specimens, the behavior of the 0-week knits was as expected. Similar break forces for 4-ply knits with equal knitting properties were reported earlier by Kellomäki,<sup>9</sup> although the strain property of our knit was  $\sim 17\%$  higher. The higher strain values are influenced by the knit density, which was lower in our study.

Increasing the number of needles in the tubular weft knit statistically significantly increased the measured tensile load. Increasing the number of single filaments in fibers in the tubular weft knit has a statistically significant effect on tensile load of certain structures; the only statistically insignificant difference was indicated between 8F-9Nb and 4F-9Nb knits. Higher numbers of filaments in fibers also affect the knitting properties. For a chosen needle and needle bed type, there is a maximum number

**Table 4.** Shelf life data of the mechanical tests for all knitted samples

	Months	Maximum Load (N)		Load/ <sub>1/2</sub> Loop (N)		Strain at Max Load (%)		Youngs Modulus (MPa)	
		Mean	StDev	Mean	StDev	Mean	StDev	Mean	StDev
8F-9Ns	0	91.7	4.3	5.1	0.2	39.2	2.3	23.9	1.5
	6	101.3	6.8	5.6	0.4	41.7	4.8	17.7	0.9
	12	100.7	6.6	5.6	0.4	40.7	3.9	19.5	2.1
	18	100.2	2.9	5.6	0.2	41.4	3.0	19.8	1.1
	24	96.7	4.1	5.4	0.2	43.3	3.6	20.1	1.5
	30	97.0	5.8	5.4	0.3	41.0	6.4	21.6	2.3
	36	103.7	2.6	5.8	0.1	44.4	2.5	21.2	1.3
8F-9Nb	0	96.6	10.1	5.4	0.6	33.4	4.3	40.3	4.3
	6	95.3	7.1	5.3	0.4	31.7	5.4	24.2	5.3
	12	96.7	6.4	5.4	0.4	35.2	5.9	26.1	0.7
	18	96.9	11.5	5.4	0.6	37.7	3.0	25.8	2.7
	24	96.9	4.5	5.4	0.2	40.0	4.2	23.1	0.4
	30	99.4	4.9	5.5	0.3	37.2	2.2	31.3	2.2
	36	91.5	7.0	5.1	0.4	37.2	6.7	29.6	1.6
4F-9Ns	0	89.3	8.2	5.0	0.5	39.1	7.7	24.3	1.3
	6	94.2	6.9	5.2	0.4	37.4	5.0	22.0	2.1
	12	80.3	8.6	4.5	0.5	28.5	5.9	22.6	1.0
	18	86.2	8.2	4.8	0.5	31.7	3.1	22.7	3.7
	24	81.1	6.4	4.5	0.4	29.6	4.7	22.2	4.5
	30	85.4	3.5	4.7	0.2	31.1	2.9	24.4	1.6
	36	81.2	4.8	4.5	0.3	31.3	4.5	23.4	4.5
4F-9Ns	0	67.8	3.1	3.8	0.2	35.9	2.5	18.4	1.0
	6	73.7	6.6	4.1	0.4	36.0	3.6	15.7	1.5
	12	71.9	2.8	4.0	0.2	37.6	5.5	14.7	3.1
	18	70.8	8.5	3.9	0.5	34.7	6.1	15.7	1.7
	24	73.0	4.2	4.1	0.2	38.5	4.9	15.2	2.8
	30	71.8	3.9	4.0	0.2	34.8	2.0	17.2	1.7
	36	70.8	3.8	3.9	0.2	36.1	3.9	17.6	0.6
4F-19Ns	0	127.2	18.6	3.3	0.5	44.1	4.7	11.4	0.6
	6	138.7	21.9	3.7	0.6	44.5	6.8	9.9	0.5
	12	131.7	4.6	3.5	0.1	45.4	4.0	9.4	0.9
	18	141.4	11.4	3.7	0.3	44.1	3.8	9.7	0.6
	24	119.0	12.6	3.1	0.3	41.5	4.5	8.5	0.8
	30	127.4	4.8	3.4	0.1	44.1	1.8	9.9	0.7
	36	125.7	14.0	3.3	0.4	45.9	3.2	10.3	0.4
8F-19Ns	0	186.8	20.6	4.9	0.5	41.6	5.1	16.5	0.9
	6	204.6	16.0	5.4	0.4	51.3	6.7	13.1	0.7
	12	194.6	12.6	5.1	0.3	46.7	3.9	13.4	0.8
	18	195.8	7.1	5.2	0.2	48.9	4.7	12.9	1.4
	24	195.8	10.2	5.2	0.3	52.2	7.4	11.4	0.9
	30	204.0	11.7	5.4	0.3	49.5	5.7	13.8	0.9
	36	190.1	14.9	5.0	0.4	45.3	6.1	14.2	0.9

n = 5 for all the data points.



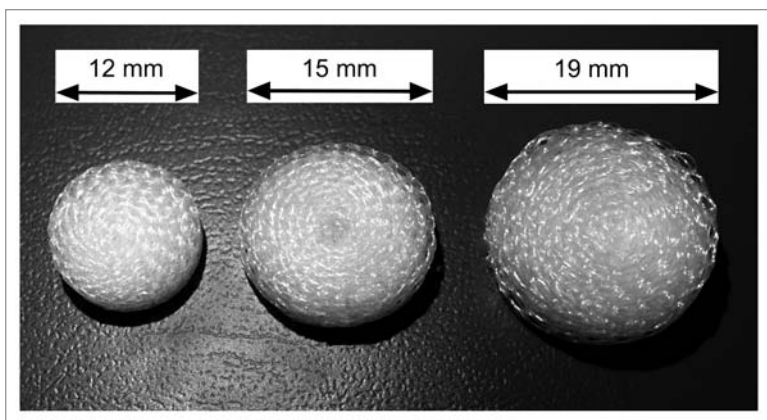
**Figure 1.** Knit info and SEM pictures of 4-filament knit of (A) 4F-9Nb (big loop size) and (B) 4F-9Ns (small loop size) after 1 week of incubation.

of filaments that can be used where the knitting procedure is optimal for a good quality knit.<sup>23</sup> The mechanical properties of weft knit fabrics are different when the wale and course directions are compared.<sup>24-26</sup> For our purposes, the wale direction is more important, since, in the tubular form, if used as such, the tensile forces are mainly induced in the wale direction. The tensile strength is higher and the extension to break lower in the wale direction. When the stretching is to the wale direction, there is a higher deformation to the wale spacing than to the course spacing.<sup>22</sup> Statistically significant loop size correlation to the tensile strength was noticed more clearly with the 4-ply knit; the longer the loop length, the higher the tensile strength. Similar behavior was noticed when using the knits as reinforcement in the composite structures.<sup>27</sup> The physical properties of the weft knit fabrics differ when the wale and/or course dimensions change, and the elastic recovery, in particular, in the wale direction is higher compared to the course direction.<sup>24</sup> By altering loop size, wale and course properties, it is possible to affect the porosity of the single jersey tubular knit, thus altering the penetration ability of liquids and gases.

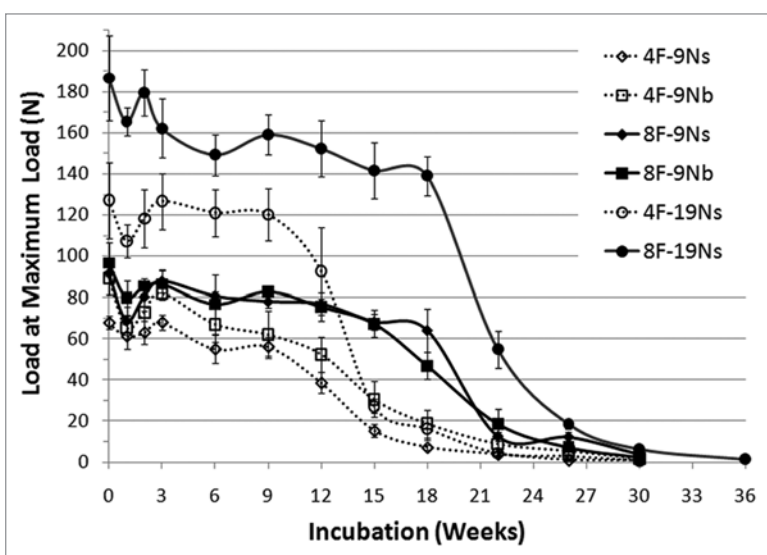
The 8-ply fiber lost 15–16% of its mechanical properties (load/<sub>1/2</sub> loop) during the 9-week hydrolysis, whereas 4-ply fiber lost 4–30%. Although they underwent similar processing conditions, the retention time was longer for the 4-ply fiber. So, in the process, there is most likely a higher monomer content in the 4-ply fiber. Based on our earlier studies in reference 28, we can predict a 0.2–0.3 wt% of lactide monomer accumulation in the fiber on the basis of the degradation behavior of this medical-grade, high i.v. PLA96. The 8-ply fiber knit retains its strength at a starting level for a longer time compared to the 4-ply knit, supporting this assumption. On the other hand, on the basis of the molecular weight studies, the 8-ply fiber lost its molecular weight faster until week 20, yet we did not observe a critical incline in the behavior compared to the 4-ply fiber. After week 20, the  $M_w$  was equalized. The monomer content differences between the fiber batches, therefore, cannot be considerable based on the behavior of  $M_w$  during the hydrolysis.<sup>29</sup> The same degradation behavior

as a function of  $M_w$  reduction for 4-ply fiber was reported by our group<sup>6</sup> for the 20-week period. It could be noticed that, when comparing the molecular weight data, the knit and the scaffold degraded similarly in vitro, so the form of the scaffold had no influence on the degradation speed. Although the knits were incubated in SBF and the scaffolds in PBS, there was no difference noticed between SBF and PBS PLLA backbone degradation, water uptake or mass loss during the first 27 weeks.<sup>30</sup> When material properties are considered, none of the structures could be preferred over the other, as the maximum load level can be achieved by increasing the amount of material and considering that knits would be used as such in suitable applications under tensile forces. If considering that minimal material usage is preferable, then the 4F-9N knits should be used, of the studied ones, when the mechanical properties are suitable for the purpose of use.

The shelf life studies showed a good uniform quality with respect to tensile properties during the whole 3-year period. As for the molecular weight properties, there is a reduction in molecular weights and viscosity and an increase in polydispersity, but there was no change in the mechanical properties and standard deviations when comparing the different knits. This only confirms that changes in molecular level that occurred during storage had no effect on the mechanical properties. It is known that the mechanical properties are not simultaneously affected, even though the molecular weight drops in the beginning of the degradation and considering that the original molecular weight is high enough, since the properties are strongly affected by the actual molecular weight, not only the original molecular weight. So, in the case of bulk PLA 96, the drastic drop in strength is also accompanied by the mass loss,<sup>31</sup> whereas in fiber form, the strength properties are more easily affected due to the subtle changes in morphology caused by the drop in molecular weight<sup>9,29</sup> prior to the loss of mass, as seen in this study, where the mass loss starts at week 24, when fiber tensile properties are already low. Only some shelf life data exists where PLA96 was studied. In a study by Pluta et al., melt-processed material was tested after aging the material for



**Figure 2.** The outlook of the 12, 15 and 19 mm scaffolds after heat treatment.



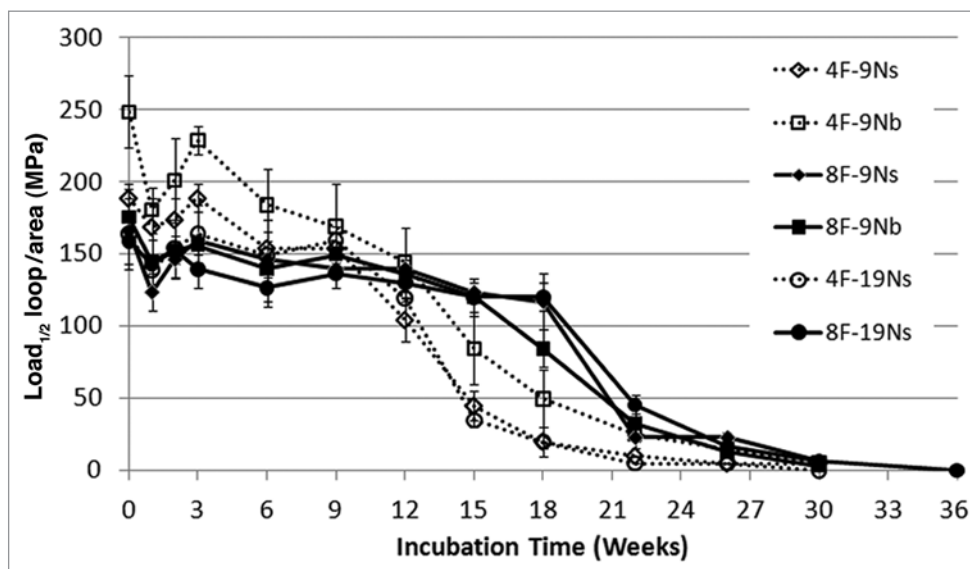
**Figure 3.** Load properties for all knitted samples ( $n = 5$  for all the data points).

1 year. Results showed no change in molecular weight properties or in polydispersity during the follow up.

It is generally known that pure PLLA loses its properties *in vivo* in a reasonable amount of time;<sup>33,34</sup> still, it is considered a slow *in vivo* degrading material due to the presence of material years after the implantation, mainly due to its high crystallinity.<sup>35,36</sup> So, the use of P(L/D)LA with 4 wt% of D-lactide in the structure has been shown to degrade faster while still retaining its properties for a long enough time to be used in operations where longer strength retention or presence is needed.<sup>17</sup> When the speed of the degradation is considered, some previous results suggest a similar<sup>30</sup> or faster<sup>9,33</sup> *in vivo* degradation compared to *in vitro* degradation for PLA polymers and PLA-based devices in the subcutaneous space of rats or rabbits. For example, the 56–99% decrease in  $M_w$  reported for PLA materials at 27 weeks in the subcutaneous mouse and rat models<sup>35,37</sup> seems to favor faster *in vivo* degradation when compared with the P(L/D)LA 96/4 fiber degradation behavior *in vitro*, where a 30–50% drop in  $M_w$  occurred during the 27-week period.<sup>9,18</sup> For a slower *in vivo* degradation,

there are indications of delayed degradation, e.g., in the case of PLLA meniscal screw, where after 3 years, the screw had 64.7% of its molecular weight left,<sup>38</sup> a result that does not correspond to animal subcutaneous models or *in vitro* models. Since many properties (e.g., material origin, processing conditions, *in vivo* environment) influence the degradation of PLA materials, there is a lot of variation in the results. The P(L/D)LA 96/4 used in our study shows similar molecular weight loss in PBS compared to other studies.<sup>6,18,31</sup> But in this study, we can show a delayed degradation in the *in vivo* compared to the *in vitro* model. The reason for this could be the increase of dense connective tissue layers. It was shown by Länsman et al. that the tissue ingrowth reaches the innermost part of the scaffold implant by 3 weeks.<sup>16</sup> Thereafter, the formation of dense connective tissue around each single filament begins, and it gets more compact after 6 weeks, with a clear increase of dense connective tissue by 12 weeks. The layers of connective tissue become denser and thicker over time, forming septae of mature dense connective tissue. The amount of vascularity decreases markedly during maturation of connective tissue. The increasing amount of mature dense connective tissue forms thickening layers around each PLDLA filament. Therefore, the layer of dense connective tissue locates in between the PLDLA filaments and vascularized looser connective tissue. These indications go together with the delayed degradation that was observed in our study, since the difference in degradation rates of *in vivo* and *in vitro* samples begins at those weeks. It is also possible that this dense connective tissue might allow the decrease of pH inside the capsule, which might slow down the degradation at the beginning of the pH decrease as suggested by Schlieker et al.<sup>39</sup>

When comparing the different scaffold types, there are no clear differences between the 12 and 15 mm scaffolds. *In vitro* 1-week compression properties of the 19 mm scaffold are lower due to the dimensions of the sample. The greater height of the sample renders the scaffold softer at the beginning compared to the other two scaffolds, since the knit structure they were made from is the same for all, and the preload of 5 N equalized the starting point for all the samples. So, the scaffolds were somewhat similar from the *in vitro* perspective. The knitted fabrics produced here are mainly intended for manufacturing a vessel to guide fibrous tissue proliferation in human body. Therefore, they have to have sufficient properties for supporting, for example, the finger joint for rheumatoid patients.<sup>17</sup> As the degradation of the material occurs and the mechanical properties vanish, it is important that the forming tissue takes up the structural forces. For this purpose, it is essential that mature, dense connective tissue can form into the sample before the sample collapses. In the rat studies,<sup>16</sup> this time has proven to be somewhere between the weeks 24–48, and we can clearly show that the effect of the maturing tissue on the compression stiffness is already seen at week 12. Although we can see a clear degradation of the material



**Figure 4.** Strength properties for all knitted samples (n = 5 for all the data points).

regarding the knit tensile properties, the properties in compression and post-operative *in vivo* show that the fiber network is still functional at week 36 and still clearly present in the tissue at week 52.<sup>16,18,19</sup> It has been shown that the same P(L/D)LA 96/4 knitted tube used here is suitable when used as a cylindrical 3D scaffold for gaining a functional metacarpophalangeal or other small joints.<sup>17,19</sup>

What it is essential to notice here is that we have shown that the fiber is not drastically affected by the knitting procedure, and the tubular knit still obtains suitable properties for a favorable time to be used for TE purposes. With parameter setting, it is possible to affect the properties of the knitted samples, although for MCP purposes, the subtle porosity differences seems to play no major part for the connective tissue forming.<sup>16</sup> Therefore, it is vital that the polymer properties and the fiber properties (amount and thickness of the filaments) are considered for even better outcome of the required tissue forming.

## Materials and Methods

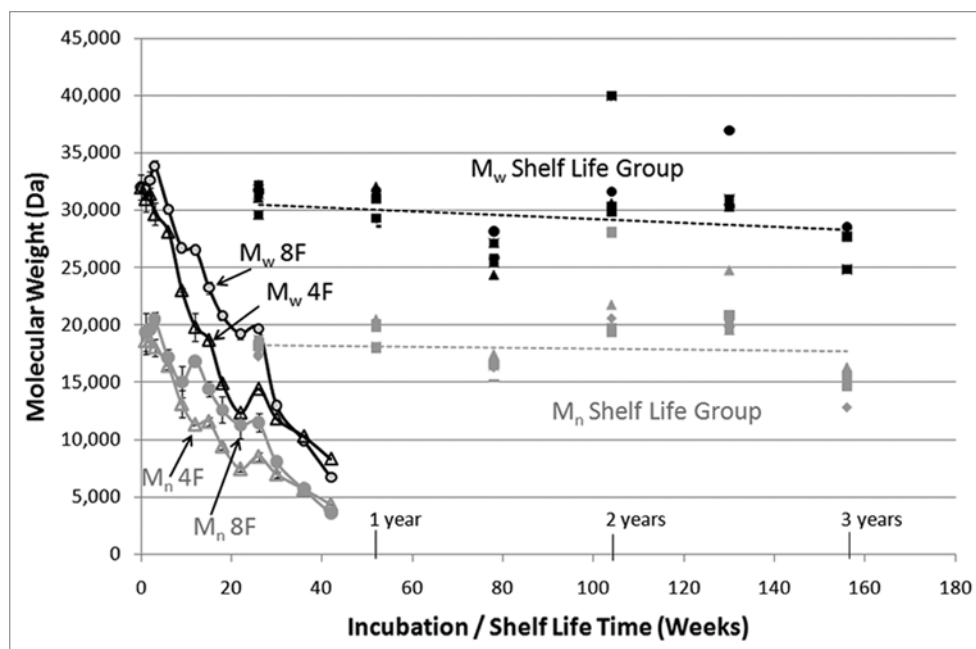
**Multifilament fibers.** Two highly purified, medical-grade poly(L/D)lactide 96/4 polymers with intrinsic viscosities of (1) 5.47 dl/g (PLA96A) and (2) 5.70 dl/g (PLA96B) (Purac Biochem, Goringhem, The Netherlands) were used for fiber manufacturing. PLA96A was melt-spun into multifilament fibers (4- and 8-ply), and PLA96B was processed to 4-ply fibers (similar to PLA96A), and these fibers were only used to produce scaffolds. In all cases, Gimac microextruder (Gimac, Gastronno, Italy) with a screw diameter of 12 mm was used. The single orifice diameter in the nozzles was 0.4 mm. The nozzle temperature was 267°C for both batches. The extruder retention time for the 4-ply fiber was 360 s, and for the 8-ply fiber, 300 s. The fibers were hot drawn using three caterpillars (draw ratio 4.5) and three IR ovens

(oven temperatures 95/115/120°C). Fibers were further used for knitting and scaffold manufacturing.

**Single jersey knits.** Both multifilament fiber batches of PLA96A were knitted to a tubular single jersey knit using a circular knitting machine equipped with a ½" needle cylinder (Elha R-1S, Textilmaschinenfabrik Harry Lucas GmbH, Neumünster, Germany). Parameters in the process were the number of the latch needles and the loop size (length of the one continuous loop) that sets the values to wales (vertical columns) and courses (horizontal rows) (Fig. 1). The samples, their knitting parameters and properties are shown in Table 1. Knits were cut to samples of 70 mm in length each, and the ends were heat sealed to prevent the loop running.

**Porous 3D cylindrical scaffolds.** The cylindrical scaffolds were manufactured from the 4F-9Ns knit made of PLA96B 4-ply multifilament fibers. The knits were cut to size according to the requested size of the ready scaffold (Table 2) and rolled into the cylindrical shape. The end of the knit was fixed to the roll with a biodegradable "glue" prepared by dissolving poly(L/DL)lactide 70/30 polymer into acetone. Scaffolds were heat treated at 70°C/15 min in a mold to obtain the desired shape (Fig. 2). The final average porosity of the scaffolds was measured using cone-beam computed tomography (ProMax 3D s, Planmeca, Helsinki, Finland).

**In vitro and in vivo.** Prior to *in vitro* incubation or *in vivo* implantation, the fibers, knits and cylindrical scaffolds were washed with 95% ethanol for 3 minutes in a laboratory-scale ultrasonic washer, dried in vacuum for 24 hours, then packed in vacuum sealed bags (inner layer bag PE, outer layer bag Aluminum/PE for UV-protection) and sterilized by  $\gamma$ -irradiation using a commercial procedure, where the minimum dose was set to 25 kGy. The fibers were incubated for periods of 0, 1, 2, 3, 6, 9, 12, 15, 18, 22, 26, 30, 36 and 42 weeks at 36.5°C in simulated



**Figure 5.** Molecular weight properties of the yarns and shelf life samples.

body fluid<sup>40</sup> (SBF) at pH 7.25. The buffer solution was changed and the pH measured at 21°C fortnightly.

The cylindrical samples were incubated for periods of 0, 1, 2, 6, 10, 16, 15, 18, 20, 24, 28, 32, 36, 42 and 48 weeks at 36.5°C at phosphate buffered saline (PBS) pH 7.25. The specimen mass/buffer solution ratio was, on average, 7.4 g/100 ml. The buffer solution was changed and the pH measured at 21°C fortnightly.

The 15 x 3.5 mm scaffolds were implanted subcutaneously into rats for periods of 3, 12, 24, 48 and 52 weeks. These samples were a part of the study by Länsmän et al., and the procedure is previously explained in detail in reference 16.

**Shelf life.** PLA96 knits were cut to size, washed, dried, packed and  $\gamma$ -irradiated (as described above). Sample packages were placed on the laboratory shelf in ambient room temperature and under normal (atmospheric) pressure to ensure the storage time. Samples were periodically tested (tensile test, molecular weight characterization) after 0.5, 1, 1.5, 2, 2.5 and 3 years.

**Material characterization.** Number of average molecular weight ( $M_n$ ), weight average molecular weight ( $M_w$ ), intrinsic viscosity (i.v.) and polydispersity (PD) were measured by gel permeation chromatography (GPC) relative to narrow polystyrene standards. GPC consisted of Waters 410 RI differential refractometer detector and Waters 515 HPLC pump (Waters, Milford, MA USA). The GPC columns were PL gel 5  $\mu$ m Guard and 2 PL gel 5  $\mu$ m mixed-C. Injection volume was 150  $\mu$ l, and the flow rate of eluent was 1 ml/min. Calibration was performed using monodisperse polystyrene standards, applying Mark-Houwink parameters for PS ( $K = 1.12 \times 10^{-4}$  and  $a = 0.73$ ). The samples were dissolved in 0.1% w/v solutions in chloroform at room temperature.

Tensile properties of the fibers and knits samples were tested using Instron 4411 Materials Testing Machine (Instron Ltd., High Wycombe, England). Crosshead speed was 30 mm/min and gauge length was 20 mm. The half loop force was calculated as:

$$\text{Load}_{\frac{1}{2}\text{loop}} = \text{Maximum load} / (2 \times \text{needle count})$$

Load <sub>$\frac{1}{2}$ loop</sub> refers to the load in the half loop, i.e., in the 4-ply or 8-ply fiber used in the knitting.

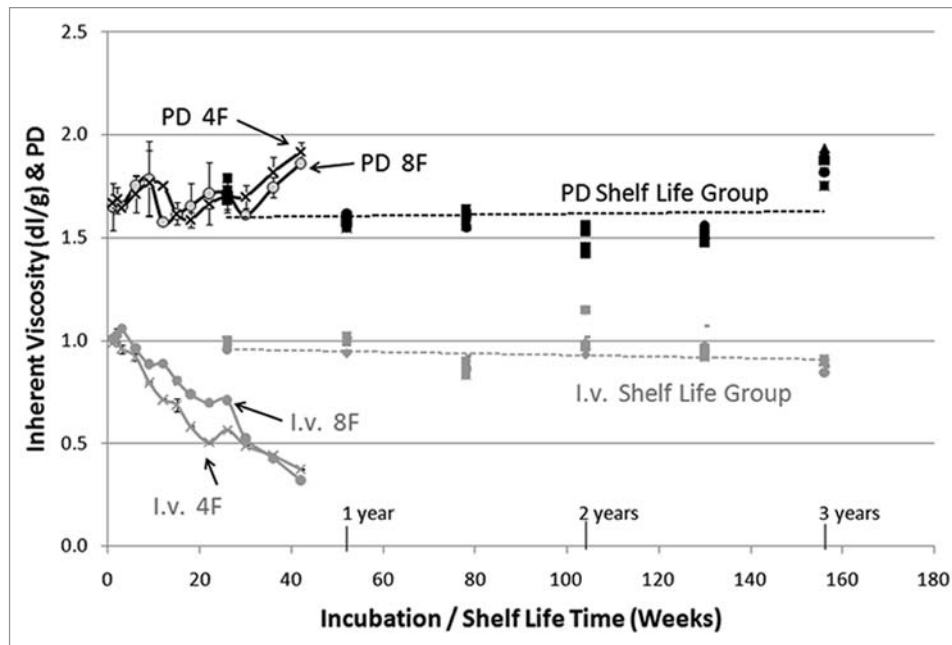
Compression properties of the cylindrical scaffolds were tested using a Lloyd LR 30K Materials Testing Machine (Lloyd Instruments Ltd., Fareham, England). Crosshead speed was 1 mm/min. Specimens were compressed between polished stainless steel, plated, and 5 N preload was applied to minimize the influence of the rough surfaces of the scaffolds, after which the new zero/starting point was set. The scaffolds were tested until 2 mm extension was reached (50–67% thickness change). Initial compression results were measured on dry specimens, and after in vitro hydrolysis, wet specimens were tested.

After the sacrifice, samples were prepared by removing the external connective tissue formed outside the scaffold. The tissue grown into the porous scaffold and the scaffold itself were left untouched. Samples were placed in the saline and tested within 24 hours from sacrifice. The above testing procedure was also used for the in vivo scaffolds.

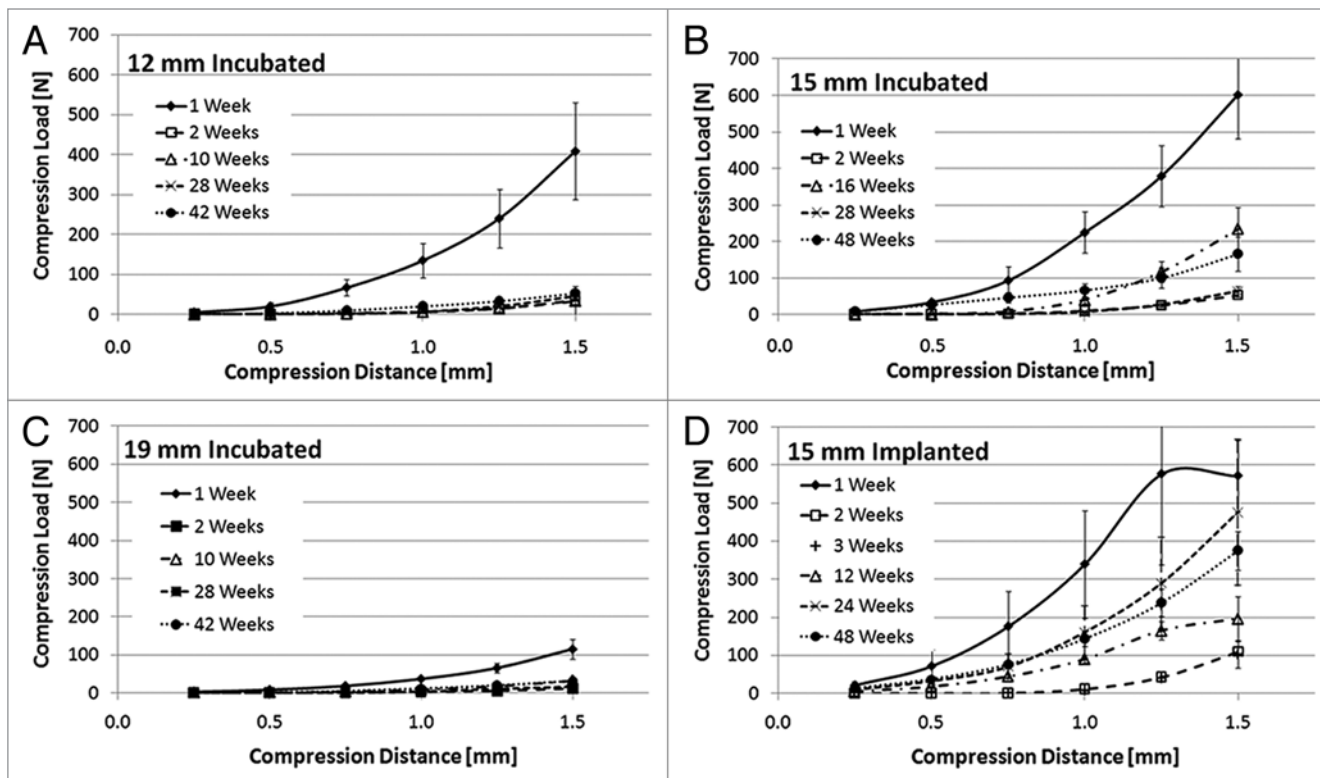
## Conclusions

The entirety of the production cycle, from raw material to an in vivo scaffold, was covered. From biodegradable PLA96 polymer





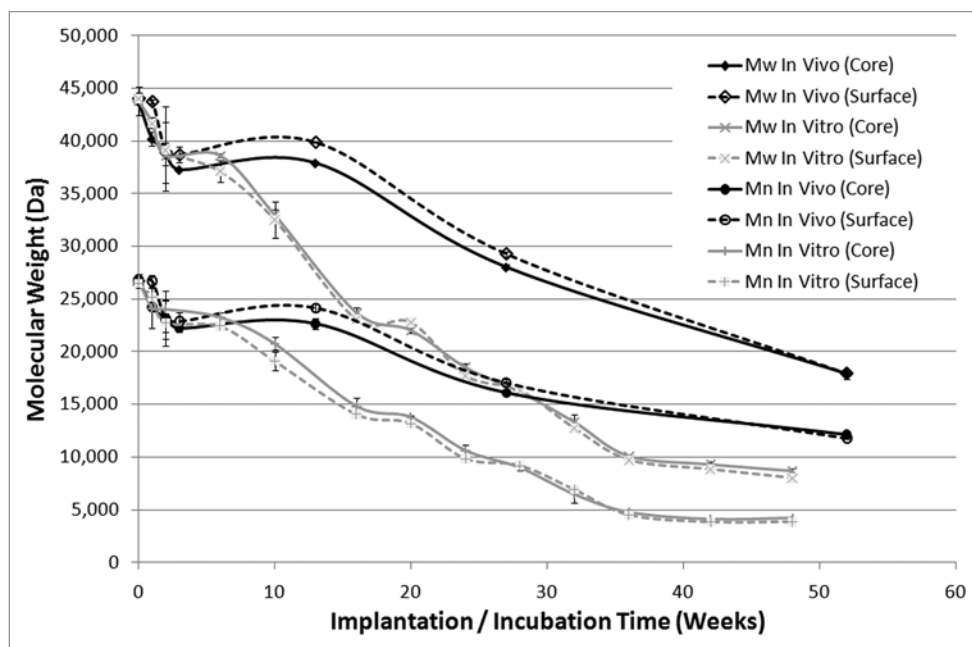
**Figure 6.** Viscosity and polydispersity data of the yarns and shelf life samples ( $n = 2$  for all data points).



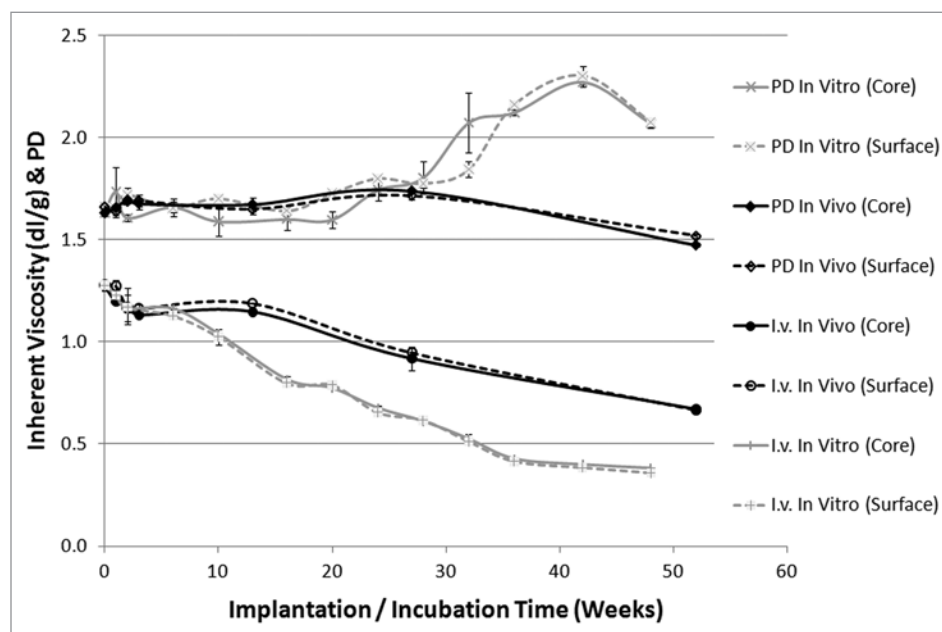
**Figure 7.** In vitro mechanical data for (A) 12, (B) 15 and (C) 19 mm scaffolds and (D) in vivo data for 15 mm scaffold ( $n = 3$  for all in vitro data points,  $n = 5$  for all in vivo data points except for 1 and 2 week data points  $n = 4$ ).

fibers, different knit types were prepared. Since these constructions are interesting for further applications, the properties of those knitted samples were tested in vitro as well as in 3-year shelf life (storage time, aging) tests. We confirmed that the

mechanical integrity is valid even after 3-year restoration in a controlled atmosphere and at an ambient temperature for the  $\gamma$ -irradiated samples when they were properly packed for storage. The polymer morphology slightly changed during the final



**Figure 8.** Molecular weight data of the 15 mm scaffold in vivo and in vitro series (n = 2 for all data points).



**Figure 9.** Polydispersity and viscosity data of the 15 mm scaffold in vivo and in vitro series (n = 2 for all data points).

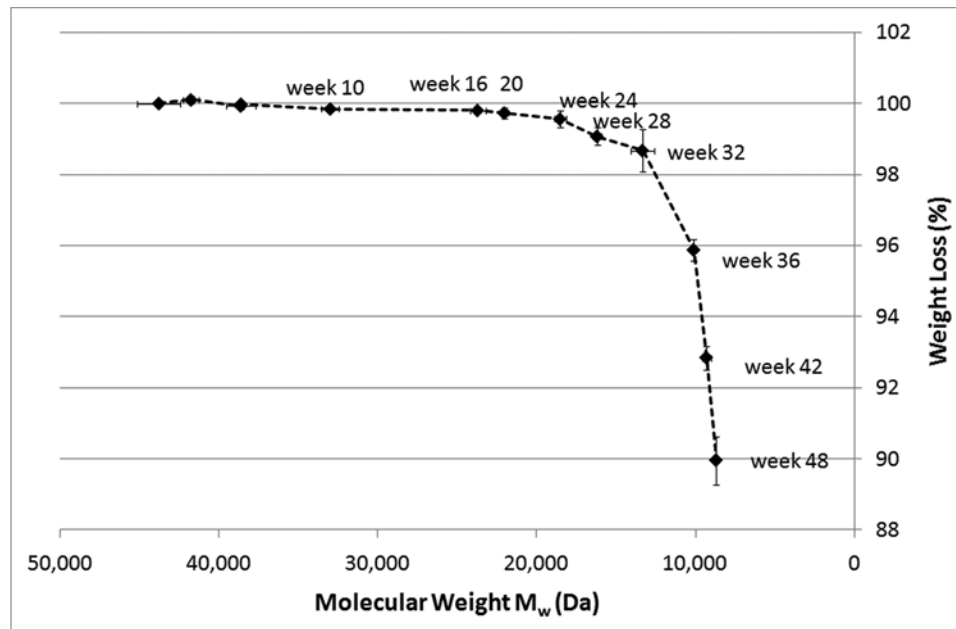
6 months of this storage period, although it had no influence on the mechanical properties. We showed that the degradation characteristics are similar when comparing the knits and the scaffolds. Furthermore, we showed that the weakened mechanical properties resulting from the immersion/implantation of the scaffolds prepared from these knits are enhanced by the ingrown tissue, which actually renders mechanical strength to the scaffold in vivo, thus making the structure a truly tissue-engineered composite. These indications are important when commercializing the structures and planning the use of such scaffolds.

#### Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

#### Acknowledgments

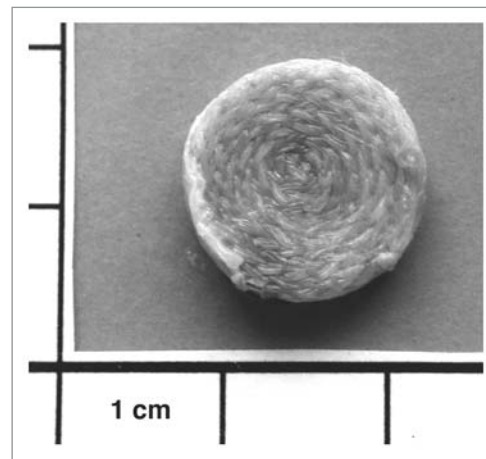
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**Figure 10.** Weight loss data ( $n = 3$ ) plotted against the  $M_w$  ( $n = 2$ ) during the 15 mm scaffold in vitro incubation.

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**Figure 11.** The scaffold after 52 weeks of implantation.

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