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Studies of P(L/D)LA 96/4 non-woven scaffolds and fibres; properties, wettability and cell spreading before and after intrusive treatment methods

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Abstract Poly(L/D)lactide 96/4 fibres with diameters of 50 and 80 μm were produced. The smaller diameter fibres were carded and needle punched to form a non-woven mat. Fibres and non-woven mats were hydrolysed for a period of 20 weeks. Fibres and pressed non-woven discs were treated with low-temperature oxygen plasma and alkaline KOH hydrolysis and ethanol washing was used as a reference treatment. The non-wovens lost 50% of their tear strength after 8 weeks in vitro while the fibres still retained 65% tensile strength after 20 weeks. Hydrolysation time in KOH, treatment time and power settings of the oxygen plasma were all directly proportional to the mechanical properties of the fibres. Increasing time (and power) resulted in lower tensile properties. Rapid wetting of the scaffolds was achieved by oxygen plasma, KOH hydrolysation and ethanol washing. Cell culturing using fibroblast cell line was carried out for the treated and non-treated non-woven scaffolds. In terms of adhesion and the spreading of the cells into the scaffold, best results after 3-day culturing were obtained for the oxygen plasma treated scaffolds.

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Introduction

Tissue engineering (TE) is one of the most challenging fields in biomaterial science as is the material technical means needed to achieve the foundation into which the cells can grow and form tissue of the desired shape and dynamics. Fibres produced from poly(L-lactic acid) (PLLA), its copolymers (PLDLA), polyglycolide (PGA), and their copolymer (PLGA) have been used as a matrix material for constructing various fabrics such as scaffolds for TE [1–4]. Scaffolds produced from the fibres should be highly porous, with a large surface area, suitable pore size and a highly interconnected network of pores [5]. Scaffold properties should also lead to a homogenous seeding density not only on the surface but also inside the scaffold [6]. The advantage in using fibre-based scaffolds such as non-woven mats over salt-leached or solvent-cast scaffolds is their high porosity and a porous network without additional chemicals; the downside is the uneven distribution of pores. Since polylactide based materials were first used as biomaterials, hydrofobicity has been an important issue. Several techniques have been used to overcome this problem and some of them, such as glow discharge gas plasma treatment [7–9] and alkaline hydrolysis [10], have shown promising results for the bulk and the 3D scaffold surfaces.

Our main objective was to manufacture non-woven scaffolds and to achieve good wettability to the scaffolds without the use of chemicals during the manufacturing process. These fabrics could be used for cell culturing purposes and also as preform for manufacturing more complex scaffold shapes. Post-treatment of the scaffolds requires deep penetration of the reacting agent, hence two methods were chosen for comparison: chemical aging that has previously shown good results when used with PHB/HV [11] and a physical low-temperature plasma treatment method. The effects of the two different surface treatment methods on the properties of highly oriented P(L/D)LA 96/4 fibres and 3D non-woven scaffolds manufactured from these fibres were studied. The influence of increased wettability and porosity of the 3D scaffolds to the adhesion of the seeded fibroblasts was examined.

Materials and methods

Fibre and scaffold manufacturing

Medical grade polymer poly(L/D)lactide 96/4 with intrinsic viscosity of 5.48 dl/g (Purac Biochem, Goringhem, The Netherlands) was used for fibre manufacturing. Polymer was melt-spun into multi-filament fibres (4- and 12-ply), using Gimac microextruder (Gimac, Gastronno, Italy) with screw diameter 12 mm. The fibres were drawn using caterpillars and ovens. The fibres were cut to staple fibres, carded and needle punched into non-woven (NW) mats. NW was compressed at a temperature of 85°C with restrictors of 1.0 mm to produce scaffolds (NWP) of ~1.0 mm thickness. The thickness of the NW prior to compression was 3–4 mm. The various compositions are shown in Table 1 and the treatments performed on the samples are presented in Table 2.

Plasma treatment

The plain fibres and the NWP Plasma scaffolds were plasma treated. Low-temperature plasma was applied using Plasma Prep 5 instrumentation (GaLa instrumente, Bad Schmalmach, Germany). The chamber was evacuated three times, adding oxygen between evacuations and finally the atmosphere was allowed to settle at 10 Pa. Frequency of 200 kHz was applied for defined periods of time from 0 to 300 s, with power settings of 50, 70 and 100 W. After plasma treatment, the oxygen atmosphere was held for an additional 5 min after which the samples were placed in airtight bags.

Table 1 Manufacturing characteristics of the fibres and non-wovens

Ultimate form	i.v. (dl/g)	Filaments per die	Porosity (%)	Single fibre diameter (μm)	Draw ratio
Fibre	5.48	4	–	80	4.6
NW	5.48	12	76–90	48–50	5
NWP	5.48	12	61–80	48–50	5

NW, non-woven; NWP, non-woven compressed to –1.0 mm thickness

Table 2 Treatments for the different samples

	Treatment times/(power)			
	Plasma	KOH	Ethanol washed	In vitro
Fibre	–	–	–	In vitro-hydrolyzation
Fibre	1–5 min/50 W	–	–	–
Fibre	1–3 min/70 W	–	–	–
Fibre	1 min/100 W	–	–	–
Fibre	–	5–240 min	–	–
NW	–	–	–	–
NW E	–	–	2 × 20 min	In vitro-hydrolyzation
NW E	–	–	2 × 20 min	In vitro-fibroblasts
NWP	–	–		–
NWP E	–	–	2 × 20 min	In vitro-fibroblasts
NWP Plasma	All used times	–	–	–
NWP Plasma	2 min/50 W	–	2 × 20 min	In vitro-fibroblasts
NWP KOH	–	All used times	–	–
NWP KOH	–	20 min	2 × 20 min	In vitro-fibroblasts

Chemical treatments

To remove any impurities caused by needle punching and carding, ethanol washing for NW E, NWP E, NWP KOH and NWP Plasma fabrics was performed prior to other treatments for a period of 2×20 min in ultrasound wash. The samples were dried in a vacuum at room temperature.

To modulate the surface properties of the fibres and the NWP KOH fabrics, the samples were subjected to alkaline hydrolysis at room temperature using 2.5 M KOH in a solution of methanol 50 vol.%/water 50 vol.% for time periods of 5, 20, 60 and 240 min. After hydrolysis the samples were dried in a laminar flow hood for 2 days.

In vitro

Prior to incubation the fibres and NW E were sterilised by gamma irradiation 25 kGy (Willy Rüsch AG, Germany). No other treatment was made to the set of in vitro fibres. The fibres were incubated for periods of 0, 1, 2, 3, 4, 8, 13, 16, and 20 weeks at 37°C in phosphate buffer solution (PBS, 3.48 g/dm^3 Na_2HPO_4–0.755 g/dm^3 NaH_2PO_4–5.9 g/dm^3 NaCl buffered saline) at pH 7.4. NW E mats were also incubated in PBS for 0, 2, 8, 16 and 20 weeks. The buffer solution was changed fortnightly.

Material characterisation

Molecular weights (M_n and M_w) 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 PLgel 5 μm Guard and 2 PL gel 5 μm mixed-C. Injection volume was 150 μl 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.

The differential scanning calorimeter (DSC) TAinstruments Q1000 (TAinstruments, New Castle, DE, USA) was used for thermal characterisation. The samples were heated from 20°C to 200°C at a rate of 20°C/min and after rapid cooling the heating procedure was repeated. Melting temperatures (T_m) were determined from the melting peak of the second heating; glass transition temperatures (T_g) were determined from the second heating and the crystallinity (X_c) of the samples was determined from the melting enthalpy using 93.7 J/g as the melting endotherm of 100% PLLA [12]. Indium was used for standard calibration.

Tensile properties of the fibres and tear strength of the NW E were tested using Instron 4411 Materials Testing Machine (Instron Ltd, High Wycombe, England). The fibre testing crosshead speed was 30 mm/min and gauge length was 50 mm, for NW E 100 mm/min and 40 mm. For the NW E testing, tear test samples (70 mm × 40 mm with a cut of 50 mm long in the middle of the shorter edge) were used. To enable comparison between the samples, tear force was divided by mass of the sample to compensate for differences in porosity.

Surface characterisation

XPS analysis was performed using an ESCALAB 200A (VG Scientific, West Sussex, UK) with PISCES software for data acquisition and analysis. For analysis, an achromatic Al (Kα) X-ray source operating at 15 kV (300 W) was used. The spectrometer, calibrated with reference to Ag 3d5/2 (368.27 eV), was operated in CAE mode with 20 eV pass energy. The take-off angle used was 90°. Data acquisition was performed at a pressure lower than 10^{-6} Pa. The value of 285.0 eV of the hydrocarbon C1s core level was used as a calibration for the absolute energy scale. Overlapping peaks were resolved into their individual components using XPSPEAK 4.1 software.

Rapid water uptake data was obtained by measuring the weight of the dry scaffold, then inserting it into a distilled water bath for a period of 5 s. After removal it was again weighed. Water uptake was calculated as a percentage of the increase in weight over the dry scaffold.

Cell culturing

Cell culturing was performed to assess the influence of the several surface modification treatments on the cells' adhesion to the treated scaffolds. The samples used were NW E, NWP E, NWP Plasma and NWP KOH scaffolds. NW E and NWP E were used as references. The scaffolds were round disks with a diameter of 10 mm. The NW E scaffolds were gamma irradiated while the rest of the scaffolds were EtO sterilised. The scaffolds were seeded with a cell suspension of mouse fibroblasts (cell line L929) and observed under light microscope (LM) and scanning electron microscope (SEM), after seeding (2 h) and after 3 days of culture.

The adherent cells were enzymatically released using 0.25% Trypsin-EDTA solution (T4049, Sigma, St.Louis, USA), concentrated by centrifugation at 1,200 rpm for 5 min and re-suspended in Dulbecco's Modified Eagle Medium (DMEM, D5523, Sigma, St.Louis, USA) supplemented with 10% fetal bovine serum (S0115, Biochrome AG, Berlin, Germany) and 1% of antibiotics solution (A5955, Sigma, St.Louis, USA). The scaffolds ($n = 3$ for each culturing period) were subsequently placed in non-adherent 12-well plates and each scaffold was seeded with 100 µl of a cell suspension at a concentration of 300×10^4 cells/ml and incubated (in a humidified atmosphere with 5% CO_2 at 37°C) for 2 h. Tissue culture polystyrene wells were used as controls. After this period, a group of scaffolds was retrieved for analysis and another one was transferred to new adherent 12-well plates and cultured up to day 3. At the end of each culture period (2 h and 3 days), the cell/scaffold constructs were rinsed with phosphate-buffered saline (PBS-P4417, Sigma , St.Louis, USA) and fixed in a solution of 2.5% gluteraldehyde for SEM and LM analysis. For observation under the LM (Axioplan 2, Zeiss, Germany), the seeded scaffolds were stained with a 0.4% methylene blue solution. For SEM analysis the scaffolds were subsequently dehydrated in a gradient series of ethanol solutions and sputter coated with gold (Jeol JFC 1100, Jeol, USA). The scaffolds were then observed using SEM (Leica Cambridge S360, Leica, Cambridge, UK).

Results

In vitro hydrolysation of the fibres and the NW E

The results can be seen in Table 3 and Fig. 1. Results for the oriented multifilament fibres showed that for the gamma irradiated fibres the ultimate tensile strength was 75% of initial value after 13 weeks of hydrolysation and for a period of 20 weeks the strength remained above 66% of the initial value. Elastic modulus remained above 75% throughout the study. The strain increased slightly until week 3 at the beginning of hydrolysation and remained at that level until week 20 where a slight increase was noted. Fibre diameter increased 12% during the 20 weeks of hydrolysation.

Results of the tear tests performed on the NW E in vitro samples showed that there was a drop of 50% from original strength within 8–10 weeks. After 15 weeks only 10% of original strength of NW E remained. High strain was obtained for all the NW E samples throughout the in vitro. At week 20 some of the samples retained only 1% of initial strength and there were very large differences in strain between the samples. As a reference, the measured maximum tear force of compressed samples NW P was 122 ± 43 N/g and the strain at maximum tear force was

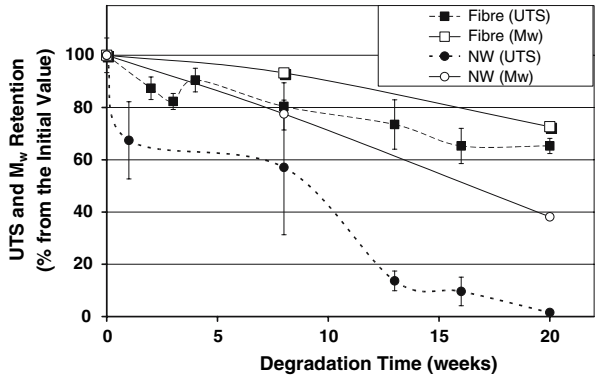

Fig. 1 Overall degradation of fibre and NW

Table 3 In vitro mechanical properties of fibre and NW

In vitro (weeks)	Fibre (multifilament fibre)			NW (non-woven)[a]	
	UTS (MPa)	Strain at Yield (%)	Elastic Modulus (GPa)	Max.Tear Force/ Weight (N/g)	Strain at Max. Tear Force (%)
0 unster.	336 ± 25	25 ± 4	7.1 ± 0.2		
0	317 ± 21	26 ± 1	7.1 ± 0.2	78 ± 12	138 ± 14
2	277 ± 12	34 ± 1	6.6 ± 0.2	41 ± 6[b]	134 ± 9[b]
3	261 ± 8	39 ± 2	5.3 ± 0.2		
4	287 ± 13	32 ± 2	6.7 ± 0.2		
8	255 ± 23	32 ± 2	6.1 ± 0.2	43 ± 13	125 ± 24
13	233 ± 22	31 ± 2	5.9 ± 0.2	9 ± 2	99 ± 6
16	207 ± 14	29 ± 1	5.7 ± 0.2	5 ± 2	96 ± 20
20	207 ± 6	36 ± 1	5.3 ± 0.2	1 ± 0	116 ± 70

$n = 5$

[a] $n = 3$

[b] 1 week

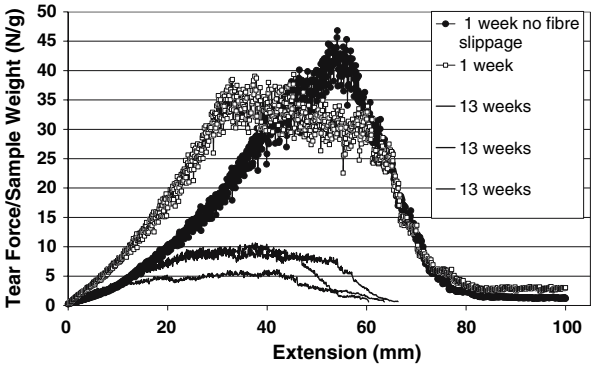

Fig. 2 Fibre slippage of NW in in vitro samples

43.8 ± 23.0%. The specific weight of the NW and NWP fabrics varied from 261 to 316 g/m^2.

Fibre slippage (needle punched entangled fibres come loose) was already seen in the first week and as hydrolysation time increased, fibre slippage in the NW E samples increased. A breaking point was observed in the 1st week samples but none was seen in the 13-week samples (Fig. 2).

Results of the molecular weight and thermal characterisation studies are presented in Table 4. A Viscosity of the polymer dropped by half during the melt processing and for the in vitro series viscosity was decreased due to gamma irradiation by a further ~70%. There was a faster reduction in molecular weight in the case of NW E compared to the fibres. A definitive increase in PD was observed at week 20 in the case of NW E, whereas there was no such increase with the fibres.

The crystallinity of the fibres was increased by gamma irradiation from 29 to 37%, it was further increased during hydrolysation to 45% after 20 weeks. In the case of NW E, crystallinity remained constant between 8 and 20 weeks. During the 20-week hydrolysation, the fibres showed a drop in T_g from 58.6°C to 54.8°C whereas T_g of NW E at 20 weeks was 50.8°C. No change in T_m was detected with the fibres during the hydrolysation (only the initial drop caused by irradiation) whereas a drop of 2°C was observed in the case of NW E from 8 to 20 weeks.

Effect of surface treatments on fibres

Oxygen plasma treatment of the oriented P(L/D)LA 96/4 fibres revealed that with power settings of 50 W the 5 min treatment time was the longest that could be carried out without significant fibre shrinkage. With 70 W the maximum treatment time was 3 min. With 100 W any treatment time resulted in significant changes and damaged the fibre. For power settings of 50 W, treatment times of 1 and 2 min showed no changes in strength or strain properties compared to untreated fibres (Fig. 3), whereas 3 min treatment time increased the strain and 5 min treatment time further increased the strain while the strength decreased. The results of the 70 W treatment showed similar behaviour at shorter time periods, which correlates with the applied power. Fibre diameter (and shrinkage) in all plasma treated samples showed plasma power and treatment dependent behaviour (Fig. 4).

Table 4 Results of the molecular and thermal studies of fibre and NW

	M_W (Da)	I.v. (dl/g)	Polydispersity (PD)	Peak T_m (°C)	Onset T_g (°C)	X_C (%)
Fibre control	130,200 ± 900	2.85 ± 0.03	1.85 ± 0.02	152.8	58.6	29
Fibre 0 week[a]	35,500 ± 500	1.04 ± 0.01	1.79 ± 0.08	155.7	56.4	37
Fibre 8 weeks[a]	33,100 ± 100	1.04 ± 0.01	1.74 ± 0.01	155.7	56.7	39
Fibre 20 weeks[a]	25,800 ± 100	0.84 ± 0.02	1.69 ± 0.01	155.1	54.8	45
Fibre plasma 50 W 1 min	132,900 ± 800	2.83 ± 0.07	1.76 ± 0.05	154.8	57.4	26
Fibre plasma 50 W 2 min	129,800 ± 1800	2.75 ± 0.04	1.82 ± 0.05	153.1	57.3	31
Fibre plasma 50 W 3 min	130,600 ± 600	2.77 ± 0.04	1.83 ± 0.06	153.6	57.7	34
Fibre plasma 50 W 5 min	126,500 ± 2400	2.76 ± 0.06	1.82 ± 0.08	153.6	58.0	32
Fibre plasma 70 W 1 min	132,900 ± 800	2.78 ± 0.08	1.77 ± 0.03	154.1	57.4	32
Fibre plasma 70 W 2 min	132,600 ± 200	2.74 ± 0.04	1.78 ± 0.01	153.1	57.8	30
Fibre plasma 70 W 3 min	128,500 ± 900	2.71 ± 0.05	1.87 ± 0.01	154.2	57.5	30
Fibre KOH 5 min	125,000 ± 1500	2.80 ± 0.01	1.03 ± 0.13	153.7	55.3	26
Fibre KOH 20 min	127,300 ± 2000	2.81 ± 0.10	1.88 ± 0.07	152.8	57.1	31
Fibre KOH 60 min	129,800 ± 400	2.85 ± 0.07	1.94 ± 0.03	152.3	56.6	44
NW 0 week[a]	26,700 ± 600	0.87 ± 0.01	1.87 ± 0.02	NA	NA	NA
NW 8 weeks[a]	20,700 ± 100	0.73 ± 0.03	1.71 ± 0.04	155.4	55.5	40
NW 20 weeks[a]	10,200 ± 100	0.41 ± 0.01	2.16 ± 0.03	153.7	50.8	41

[a] Gamma sterilised

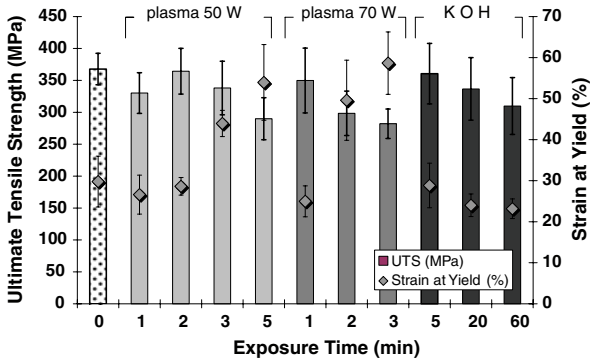

Fig. 3 Mechanical results of the treated fibres

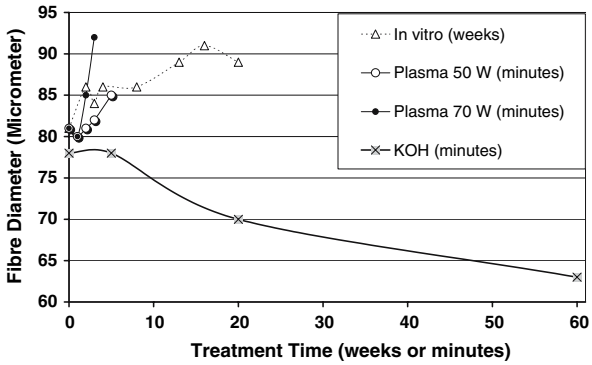

Fig. 4 Fibre dimension versus treatment and in vitro times

Treatment time of 5 min with KOH had no effect on the fibre properties or dimensions whereas both the 20 and 60 min treatment times had an effect on the strength and dimensions due to fibre dissolution. There was no difference in strain properties between the 20 or 60 min treatment times. Treatment time of 60 min showed a decrease of 38% in fibre volume and a decrease of 14% in UTS. Treatment time of 240 min resulted in dissolution of almost all the fibres and their properties could not be measured. There was no change in molecular or thermal properties among the plasma treated groups whereas in the KOH treated groups crystallinity increased along with treatment time.

Effect of surface treatments on NW

On the basis of the fibre studies, a plasma treatment power of 50 W and a time of 2 min were chosen for NWP Plasma. For NWP KOH, a 20 min treatment time was chosen. Rapid water uptake tests (Fig. 5) showed a significant decrease in water uptake when NW and NWP samples were compared. Between the compressed samples, the surface treated NWP Plasma samples doubled and the NWP KOH had 40% increase

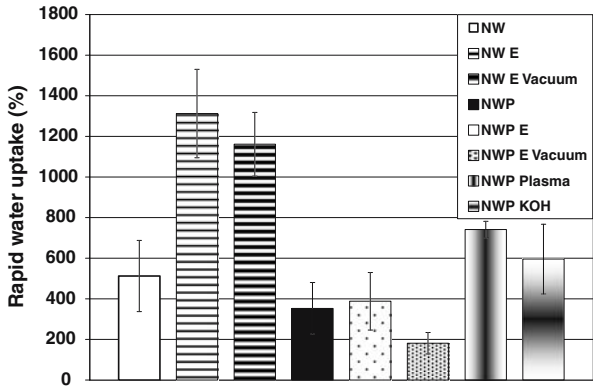

Fig. 5 Rapid water uptake results of NW and treated NWs

Table 5 XPS data of C1s envelope and atomic relation

	C1s (Area/Tot. Peak Area [%])			Atomic % relation
	C–C	C–O	C=O	O/C
NWP	56.8	24.6	19.6	0.49
NWP Plasma	63.4	25.8	13.8	0.51
NWP KOH	79.2	11.2	12.4	0.55

in the amount of water uptake compared to the NWP samples. Most of the water absorbed by the NWP samples was attached to the surface as opposed to the inside of the scaffold. The NW E and NWP E samples showed an increase in water uptake after drying in a flow cabinet. However, after vacuum drying no improvement was obtained with ethanol washing with NWP E Vacuum compared to NWP.

Surface treatments showed only a slight increase in total oxygen content (Table 5). There was a discernable decrease in content of C=O bonds with the NWP Plasma samples. Surface energy could not be calculated using the sessile drop method due to the roughness and protruding fibres on the surface of all the non-woven samples. On the untreated surfaces it was observed that fibres penetrating the contact angle drop did not break the drop. When it did occur (Table 6), total adsorption of the contact angle drop (wetting of the sample) was very fast, ranging from 80 to 300 ms.

Cell seeding

When concentrated cell medium was applied to the NWP Plasma (50 W, 2 min) and the NWP KOH (20 min) scaffolds the medium was adsorbed immediately. When seeding the NW E and NWP E (Fig. 6) scaffolds, the concentrated medium droplet remained on the surface until rest of the medium was added 2 h later.

Table 6 Contact angle testing results

	Wetting
NW & NW (gamma ster.)	No wetting
NWP	No wetting
NWP E	Total wetting[b]
NWP E (vacuum dried)	No wetting
NWP E (EtO)	No wetting
NWP Plasma[a]	Total wetting[b]
NWP Plasma (EtO)	Total wetting[b]
NWP KOH[a]	Total wetting[b]
NWP KOH (EtO)	Total wetting[b]

$n = 6$

[a] All treatment times

[b] Total adsorbtion of the drop in <260 ms

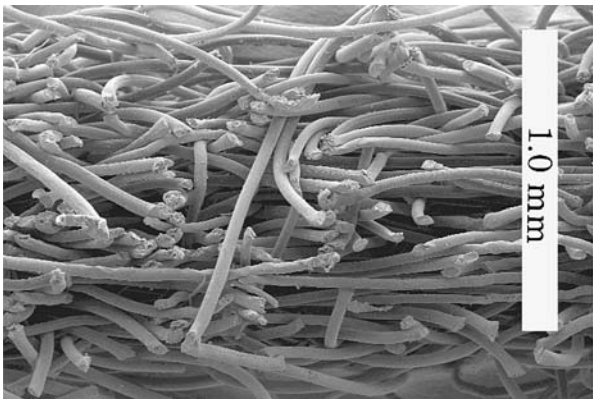

Fig. 6 SEM Picture of NWP sample from the vertical cut surface of the scaffold

After 3 days incubation (Fig. 7) it was confirmed from the SEM pictures that both the untreated NWP and ethanol washed NWP E had cells present in the confluent layer on the surface of the fibres. The cells had not penetrated very deep into the NW E and NWP E scaffolds, continuous layers of cells were found only on the uppermost part of the scaffolds. Cells were detected only on the surface area which was the same size as the incubation droplet and situated directly under the droplet. Only a few individual cells were found outside of this area. In the NWP Plasma scaffolds phenotypically viable and growing cells were found all over the scaffolds, although thicker layers of cells were to be found only in the uppermost layers of the scaffolds. In NWP KOH only a few cells, many of them dead, were found in the scaffolds after three day's incubation.

Discussion

The multifilament fibres in our study retained their UTS for 20 weeks better than the gamma irradiated PLLA monofilament fibres, poly(L/D)lactide 96/4 sutures or yarns, reported earlier [13, 14, 2]. Similar in vitro results were documented with thicker gamma irradiated 500 μm PLLA sutures for strength and strain behaviour [15]. The strength retention behaviour of PLLA multifilament fibres tested as braids [16] was similar to our multifilament fibres that were thicker; their study also showed that thicker monofilament fibres lost their strength faster than thinner multifilament fibres due to a lower orientation degree and crystallinity. Compared to the reported study [13], the fibres in our study retained strength for longer having lower crystallinity, lower intrinsic viscosity after irradiation and D-lactide in the polymer. We believe,

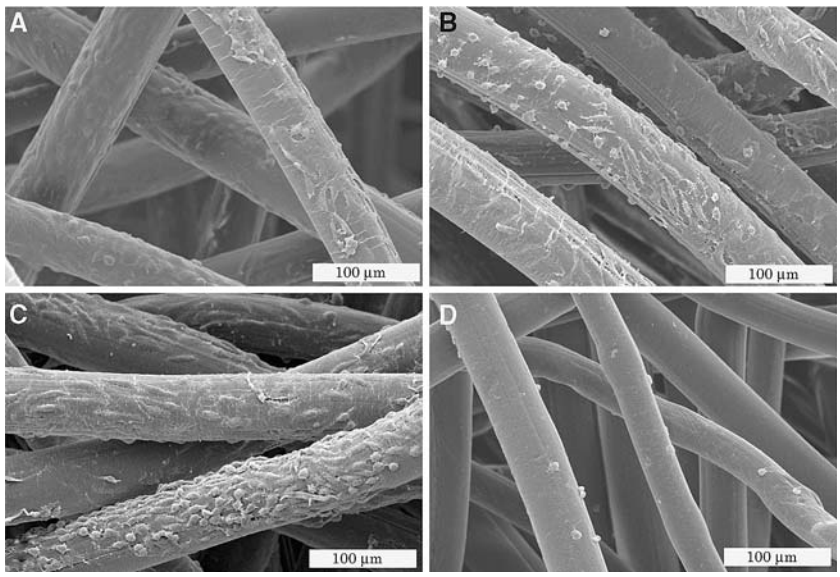

Fig. 7 SEM pictures of samples seeded with cells (fibroblast adhesion and spreading) after 3-day culturing from (**a**) NW E, (**b**) NWP E, (**c**) NWP Plasma and (**d**) NWP KOH scaffolds

therefore, that a higher degree of orientation itself is the key factor as well as monomer content [17, 18] that affects the speed of degradation in fibres. While higher crystallinity increases the strength it does not, however, decrease the strength retention speed of the fibres. When comparing our multifilament fibres to the NW fibres, processing conditions caused a greater drop in both molecular weight and viscosity that could have increased monomer content in the NW fibres thus causing faster degradation. For semi-crystalline poly (L/D)lactide 96/4,crystallinity increased, and molecular weight decreased during gamma irradiation and was further increased during the in vitro. This is consistent with other studies [13, 16, 19]. A similar decrease of ~60% in viscosity was also noticed in earlier studies of PLLA fibres [13].

A very small increase in molecular weight with oxygen plasma treated P(L/DL)LA pins has been reported [20], though this behaviour was not observed in the present study where treatment times and the used power settings were lower. A temperature increase was noticed in the chamber during the treatment and this caused a certain degree of relaxation to the fibres that was manifested as increased diameter and strain. The increase in fibre diameter caused by relaxation was proportional to the used treatment time and power. Previous studies have found no change in bending properties [20] whereas a change in tensile properties was observed in our study due to a temperature increase that has a greater effect over a shorter period of time on the thin oriented fibres than on the thicker, less oriented objects.

Tear strength test does not distinguish between the slippage of the fibres and the breakage of the entangled fibres. The slippage of the fibres can be seen in the data as a prolonged yield point in maximum break force. Slippage is an indication of asymmetrical looping and weak re-looping where the bonding fibre is forming more than one loop through the non-woven structure. This slippage can also be an advantage when considering the dynamical model. This model may have the advantage of making the non-woven adapt more easily to the in situ without loosing its maximum strength. For prolonged periods, where strength is required to last more than 10 weeks for in vitro/in vivo purposes, this non-woven may not be suitable; heavier needle punching parameters should be considered to obtain a more durable fabric. Variation in fabric weight was mainly due to pre-fabric variations caused by static electricity during carding. This variation made the fabric uneven and changed local porosity.

An increase in O/C content in XPS measurements for the plasma treated samples was previously reported for PLLA and PLGA [7, 9]. An increase of such magnitude was not observed in our studies where shorter treatment times were used. Varying results are also reported for PLLA O/C content, ranging from 0.23 to 0.55 [21, 22]. The different results show that the accuracy of the surface bound XPS analysis is dependent on surface topography. We did not detect an increase in –C–O– that could lead to hydroxyl, ether or peroxyl group incorporation, although the plasma treated scaffold itself was very hydrophilic. This was probably due to an increase in polar component on the scaffold fibres which needs to be determined.

Even with a large volume change during KOH aging, the diluted fibres had more strength left when comparing strength over the fibre cross-section area. This result is probably due to the re-crystallization of the dissolved polymer on the surface layers of the fibre. It could also be that the inner part of the fibre actually holds more crystalline structure than the surface. We believe the latter explanation to be correct because during quenching in processing, the surface cools faster thus possibly giving it a less crystalline structure.

It has been noted that the rapid attachment of cells to open network fibre structures is needed, although it was suggested that homogenous cell distribution is the problem with non-woven products [4]. In the case of our NWP Plasma treated scaffolds, fairly even distribution was achieved due to fast wetting and scaffold parameters. Variable density and an uneven distribution of fibres in some parts did not prevent the overall distribution of the cells. The authors recognise, however, that more studies are needed to verify the actual minimum density of the fibres that could act as a clot.

The NWP KOH scaffolds worked poorly in this study. It was noticed that the lowest –C–O– fraction had weaker cell adhesion [9] in the case of PLGA. This was also the case in our study, and fibre thickness was also lower in the case of NWP KOH compared to other scaffolds. It is also possible that residue potassium leads to an alkaline environment and affected cell properties and cell viability.

As seen earlier, the oxygen plasma treatment decreased the hydrophobicity of the poly(L/D)lactide 96/4 non-woven scaffolds. This enhanced the culture medium penetration to the scaffolds and may lead to an easier incubation of different types of cell into non-woven scaffolds made from poly(L/D)lactide 96/4 fibres. The influence of porosity could not be confirmed since no total wetting of the highly porous NW was achieved. Fibre diameter plays a more important role in actual attachment to the fibre. Further study is needed to determine which is the better option for tissue engineering: scaffold porosity with very thin

fibres where cells are entrapped between the fibres or a scaffold with thicker fibres where cells attach directly to the fibres.

A biodegradable non-woven structure combined with a suitable surface modification method is an interesting and feasible way to make scaffolds for tissue engineering purposes. Lactide non-woven scaffolds treated with physical low-temperature oxygen plasma could be used as potential scaffolds in further TE studies.

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