

Development and structural evaluation of poly(lactic acid) based knitted scaffold for human urinary bladder reconstruction

Bhuvanesh Gupta^a & Nilesh Revagade

Department of Textile Technology, Indian Institute of Technology, New Delhi 110 016, India

Received 12 March 2008; revised received and accepted 3 November 2008

Knitted scaffolds of poly(lactic acid) (PLA) filament spun by dry-jet-wet technique have been developed for human urinary bladder reconstruction. Three different types of samples are prepared by varying the number of ply in the PLA yarn. Flexural rigidity of PLA monofilament is found to be dependent on draw ratio. It decreases from $16.22 \times 10^{-7} \text{ kg m}^4$ to $2.74 \times 10^{-7} \text{ kg m}^4$ as the draw ratio increases from 2 to 10. The performance of the developed structures is evaluated by ball bursting technique on a tensile tester. It is observed that the knitted structure with 8 ply yarn has bursting strength of 53.7 kg with 2.5 cm extension. The cyclic loading of knitted structure has been carried out at the load half of the bursting loads. It is found that the structure gets permanently set after 5 cycles of loading. Porosity of the knitted fabric is determined by ratio of voids volume to that of the total volume of knitted fabric. The knitted structure with 8 ply yarn has 80% porosity. Pore size of the knitted structures is determined by optical microscope. The *in vitro* degradation of the PLA knittings at different pH (4.6, 7.4 and 8.0) has been carried out for a period of 20 weeks. The surface morphology was studied by scanning electron microscopy. A severe degradation of sample is observed at pH 4.6.

Keywords: Biodegradable fibre, Dry-jet-wet spinning, Poly(lactic acid), Scaffold

1 Introduction

The dramatic advances in the field of material science combined with the latest knowledge of molecular cell biology have been used to explore the novel area of biomaterials for new applications especially in 'tissue engineering'. Tissue engineering is an interdisciplinary field that applies the principles of engineering and life sciences towards the development of biological substitutes that restore, maintain or improve tissue functions¹. Langer and Vacanti¹ have described the three strategies used in tissue engineering, namely cell substitutes, tissue-inducing substances and cell placed on or within matrices. Among these, the third approach has gained most success in the tissue engineering research. It constitutes a development of biological substrates to treat the loss or dysfunction of an organ, implying some combinations of cells and scaffolds materials.

Scaffold is the material system on which the cells are allowed to seed, grow and proliferate into three dimensional ways. Scaffold is not only defined as the three-dimensional space for the formation of new tissues with appropriate structures but also may guide the development of new tissues with appropriate

function². Polymeric materials are often used as scaffolds for immobilization of biologically active molecules and living cells. Several polymers have been investigated for tissue engineering including hydroxypatite (HA), poly(α -hydroxyesters), and natural polymers, such as collagen and chitin³.

The tailoring of scaffold is not usually generic but it is always application specific⁴. Each scaffolding material or combination of materials possesses different processing requirements and varying degree of processability to form scaffolds. The key factors necessary to assess a fabrication technique for scaffold production are process conditions, accuracy of the process, repeatability and consistency of the process⁵. The most common scaffold fabrication techniques for tissue engineering are phase separation⁶⁻⁸, particulate leaching⁹⁻¹¹, freeze drying¹²⁻¹⁴, composite foams preparation^{15,16}, polymeric films modification^{17,18}, solid freeform development⁵ and textile processing techniques¹⁹⁻²². Each technique has its own advantages and drawbacks which are comprehensively discussed by Leong *et al.*⁵. Therefore, the designing and fabrication of scaffolds is done according to its application.

The minimum requirements for the scaffold material to be used for human urinary bladder are elasticity, porosity, drapability and good mechanical

^aTo whom all the correspondence should be addressed.
E-mail: bgupta@textile.iitd.ac.in

properties. In earlier attempts, the bioreceptive poly(ethylene terephthalate) (PET) films were developed where urothelial cells and smooth muscle cells were seeded for their subsequent growth into a tissue^{17,23,24}. The modified PET films were observed to be an excellent surface for urinary bladder cell culture, but had some drawbacks like porosity and elasticity. In latter attempt, the bioreceptive PET knittings were developed for the same application²⁵. The knitted surface has various advantages like very high elasticity and porosity in addition to the good mechanical properties and drapability²². However, the limitation of this bioreceptive PET knitted scaffold is its non-biodegradability. It can be overcome by replacing the biostable PET by any biodegradable polymer such as lactides, polysaccharides, etc.

The present work was therefore aimed at developing biodegradable poly(lactic acid) (PLA) knitted scaffold for human urinary bladder reconstruction. The PLA filament was developed by innovative dry-jet-wet method followed by subsequent post spinning operations. Further, weft knitting of these PLA filaments was carried out to develop the scaffold with required characteristics. The *in vitro* testing of the fabricated scaffold was carried out to evaluate its performance for its application in reconstruction of human urinary bladder.

2 Materials and Methods

2.1 Materials

PLA ($M_v = 1.5 \times 10^5$) was received from polymer laboratory, University of Uppsala, Sweden and was stored under vacuum at ambient conditions. Chloroform and methanol were supplied from Merck India. Chloroform was dried over phosphorous pentaoxide prior to the polymer dissolution.

2.2 Methods

2.2.1 Development of PLA Fibre

The dry-jet-wet technique was employed to spin the PLA filament. The spinning solution of PLA (24% w/v) was prepared by dissolving the PLA in chloroform. The spinning was carried out on a wet spinning machine fabricated by Bradford University Research Limited (Fig. 1a).

The polymer solution was extruded through the spinneret of 0.5 mm into the coagulation bath containing methanol. Both the coagulation baths had methanol as the coagulant and filament was collected using two sets of take-up rollers, both operating at the speed of 10 m/min. The air gap between the spinneret

and the coagulation bath was kept as 25 mm. The throughput rate was 0.4 g/min. The filament was collected on bobbins and then subjected to drawing process in the second step (Fig. 1b). The two-stage hot drawing of as-spun PLA monofilament was carried out to develop the PLA filament with desired properties.

2.2.2 Flexural Rigidity of PLA Monofilament

Flexural rigidity of PLA monofilament was determined by ring loop method. The loop of PLA monofilament of 7.75 cm circumference (L) was formed and loaded with 0.045 gf (M). The difference in the initial and the final diameter of loop gave the values of deflection (d). The flexural rigidity (G) was calculated by finding out the ratio of deflection to circumference and the constant (Z) based on the value of ratio, as shown below:

$$\text{Flexural rigidity } (G) = \frac{ML^2}{Z} \text{ gf cm}^2 \quad \dots (1)$$

$$= 981 \frac{ML^2}{Z} \text{ dynes cm}^2 \quad \dots (2)$$

2.2.3 Fabrication of PLA Knitting

The 2, 4 and 8 ply PLA yarns were prepared by doubling process prior to knitting. The knitting was carried out on single end weft knitting machine of the diameter 3.5 inch and gauge 14 needles/inch. The knitted fabric was tested for mechanical properties and porosity.

2.2.4 Mechanical Properties

The tensile properties of PLA knitted fabric was measured using an Instron tensile tester

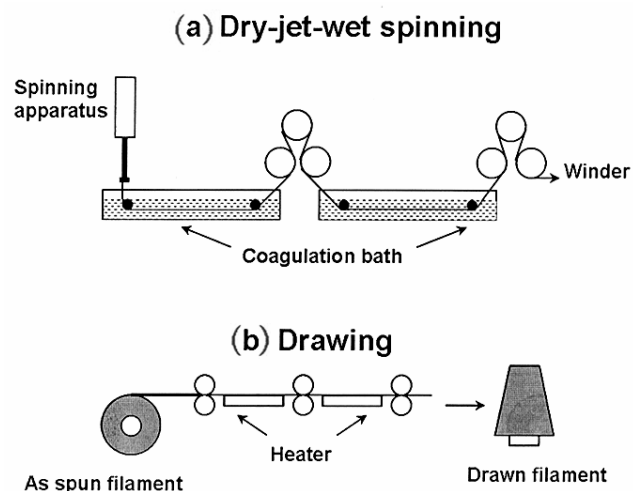


Fig. 1— Development of PLA filament by dry-jet-wet technique and post spinning process

(Model 4202). The 'ball bursting' technique was used for testing the tensile properties of the knitted fabric. All the experiments were carried out using 1000 kgf load cell and 100 mm/min rate of testing. The testing set-up is shown in Fig. 2. Two types of testing were carried out. In the first test, the maximum load to burst the fabric and the maximum extension of the fabric were observed whereas in the second test, the number of cycles was observed to set the permanent deformation in the PLA knitted fabric.

2.2.5 Porosity of Knitted Structures

Porosity of knitted structures was determined by ratio of voids volume to that of the total volume of knitted structure. The voids volume is the difference of the total volume and the polymer volume. Polymer volume was obtained by weight in terms of g/m² (gsm) of the fabric and density of the polymer (PLA density is 1.29 g/cm³). The total volume was determined by thickness and area of the fabric. Pore size of the knitted structure is determined by the optical microscope. The images of fabric samples were taken at magnification of $\times 8$. The blank area represents the pore. The blank area in between the two loops and inside the loop was measured by Leica software.

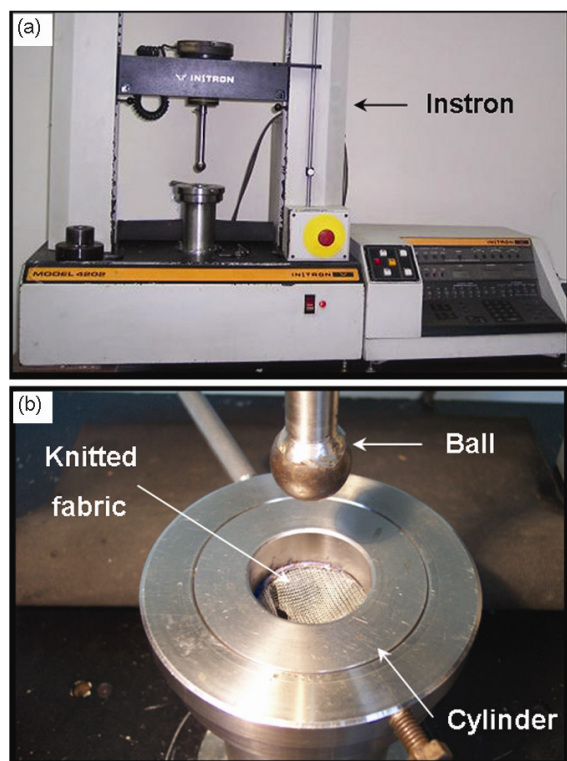


Fig. 2— (a) Testing set-up for the fabric (b) closer view of the assembly

2.2.6 In vitro Degradation Analysis by SEM

In vitro degradation of PLA knitted scaffold was carried out using three different pH (4.6, 7.4 and 8.0) at 37°C for the period of 20 weeks. The surface morphology of degraded PLA knitting was analyzed using LEO 435 VP (Leica- Oxford Link-Isis) scanning electron microscope after coating them with gold at All India Institute of Medical Sciences, New Delhi, India.

3 Results and Discussion

3.1 Spinning and Drawing of PLA Fibre

Dry-jet-wet spinning technique of PLA filament involves the process of phase inversion where the polymer in solution form gets precipitated in the non-solvent. Phase inversion process leads to the development of porous structure. Here, PLA solution was prepared in chloroform which is the best solvent for PLA and coagulation was carried out in methanol which acts as a non-solvent. The process details and the resultant filament properties have already been discussed in the previous publications^{26,27}. The filaments so developed by this process are trilobal in cross-section with tenacity ranging from 0.14 GPa to 0.60 GPa. The mechanical properties are observed to be dependent on the draw ratio, drawing temperature and heat setting temperature. The cumulative effect of these process parameters on molecular orientation and development of crystallinity in resultant filament results into filaments with different mechanical properties. The take-up speed at the spinning stage plays an important role in fibre properties development. As the take-up speed increases, the spinning stretch increases and this gives better molecular chain orientation.

Eenink *et al.*²⁸ reported the use of the PLA as-spun fibres for the drug delivery prepared by dry-wet spinning process. The use of wet spinning technique to produce the PLA fibre was also reported for biomedical application²⁹. But both the reports were limited to the fibre formation. In this work, the post spinning operations were further carried out to develop the PLA filament with desired properties. The as-spun PLA filament was drawn at different draw ratios on two stage drawing machine at drawing temperature 90°C and heat setting temperature 120°C. For the knitting, filament with draw ratio of 6 was selected having tenacity 0.49 GPa and elongation 28 %. The knittings were fabricated separately with 2, 4 and 8 ply yarns.

3.2 Flexural Rigidity

Flexural rigidity of the filament is direct indication of the bending properties of the filament which is most important property when the intended application is for knitting. Flexural rigidity is defined as the force couple required to bend a monofilament to a unit curvature. The formation of loop, size of loop and the fabric behaviour are dependent on the rigidity of the filament. The simplest way of measuring the flexural rigidity is ring-loop test. The flexural rigidity of the PLA monofilament drawn at different draw ratios was found out by the above-described process as shown in the Fig. 3. It is observed that as the draw ratio increases the flexural rigidity decreases. The bending property of the filament is one of the important factors for knitting process. Flexural rigidity is direct measurement for bending property. All the filaments drawn at different draw ratios were tested for flexural rigidity. The decrease in the flexural rigidity with the increase in

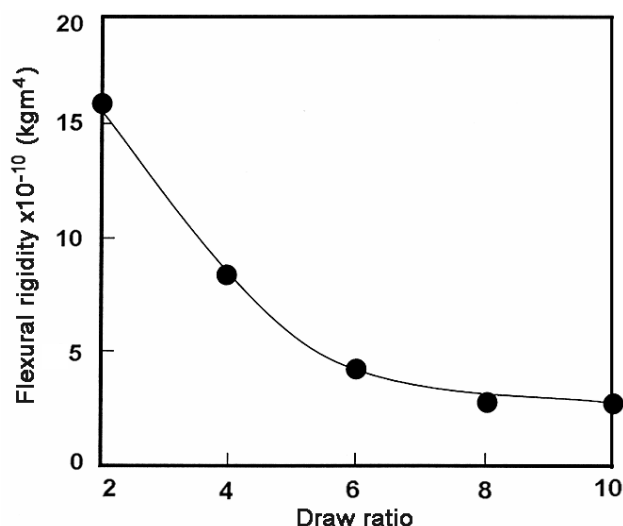


Fig. 3— Effect of draw ratio on flexural rigidity of the PLA monofilament (drawing temperature, 90°C and heat setting temperature, 120°C)

draw ratio is well predicted because as the draw ratio increases, thinning of the filament takes place which means mass per unit length decreases. It directly affects the rigidity of the filament.

3.3 Mechanical Properties

The PLA knitting was developed on single end weft knitting machine using 2, 4 and 8 ply PLA yarns. The knitted structures are shown in the Fig. 4. The loops formed are of uniform size. All the three structures were evaluated for mechanical properties and for *in vitro* degradation studies.

The ball bursting technique was employed to determine the behaviour of knitted fabric under pressure. It more or less simulates the behaviour of urinary bladder in the urine filling process. The maximum intravesical pressure on human urinary bladder wall is 40 mmHg which is equivalent to 5.3×10^{-3} MPa. The stress value obtained by bursting test for knitted fabric of 8 ply yarn is 0.33 MPa which is considerably higher than the required value. Also, the extension of the knitted fabric is found to be high. The maximum load to burst the fabric was observed to be dependent on the number of ply of yarn. For the knitting of 8 ply yarn the maximum load required to burst is 53.7 kg which is quite high as compared to 20.3 kg and 11.6 kg for 4 ply and 2 ply yarn respectively. Same trend is also observed in case of extension though the difference is not large (Fig. 5). The crack in the fabric due to bursting occurs in 'I' shape (Fig. 6).

Another type of test that was carried out to evaluate the performance of the knitted structure is cyclic loading at half load of bursting load. The intended application of knitted structure is as a scaffold material for human bladder reconstruction. That is why this test was performed to determine the deformation property of the knitting. It is observed that after 4-5 cycles of loading, the material gets

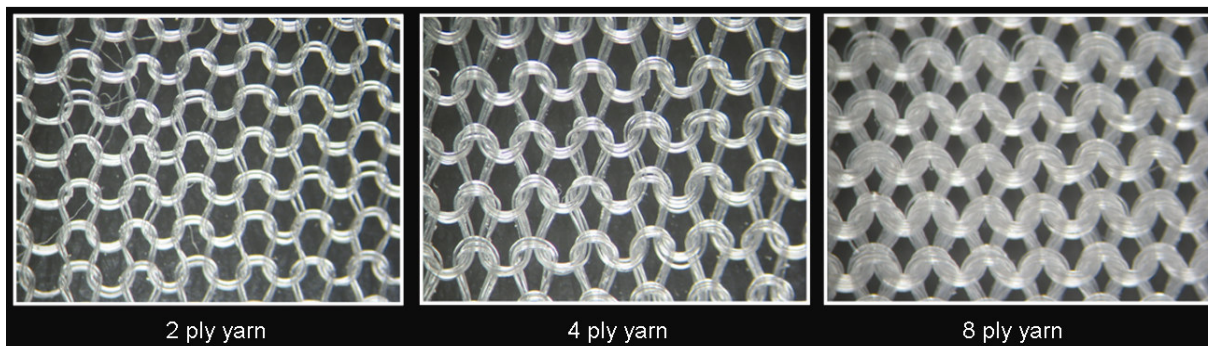


Fig. 4— Images of knitted fabrics obtained by optical microscope (magnification $\times 8$)

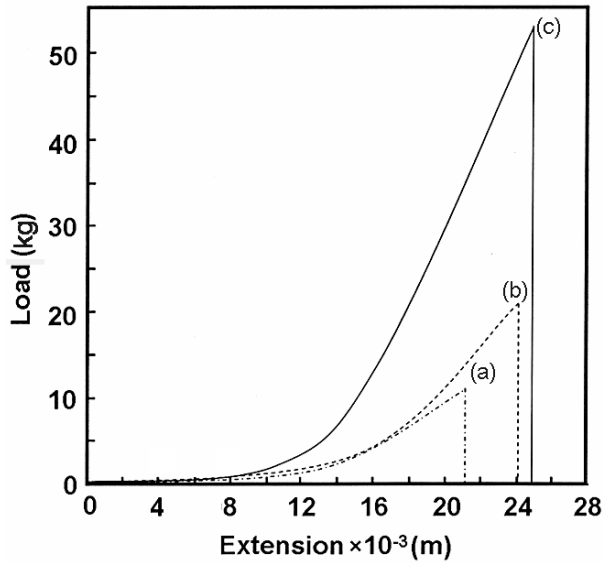


Fig. 5— Load-extension plots of knitted fabrics in ball bursting test [(a) 2 ply yarn, (b) 4 ply yarn and (c) 8 ply yarn]

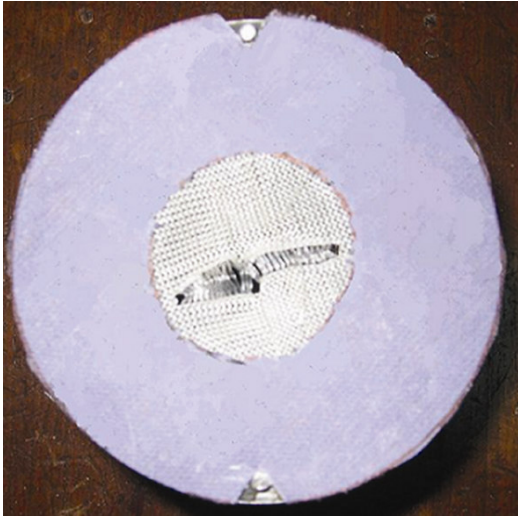


Fig. 6— Burst knitted fabric

stabilized and the load-extension curve starts repeating for further loading cycles. The residual extension in the fabric becomes almost constant after that. The cyclic load-extension curves for each type of knittings are shown in Fig. 7. In case of cyclic loading, initially fabric shows higher extension. It is because at initial cycles of loading, individual loops give the contribution towards the extension in addition to the elasticity of the yarn. After few cycles of loading, the contribution from the loops decreases due to their deformation and the residual extension is the result of the inherent extension of yarn. It is observed that after 4-5 cycles of loading the fabric gets deformed in all the three cases.

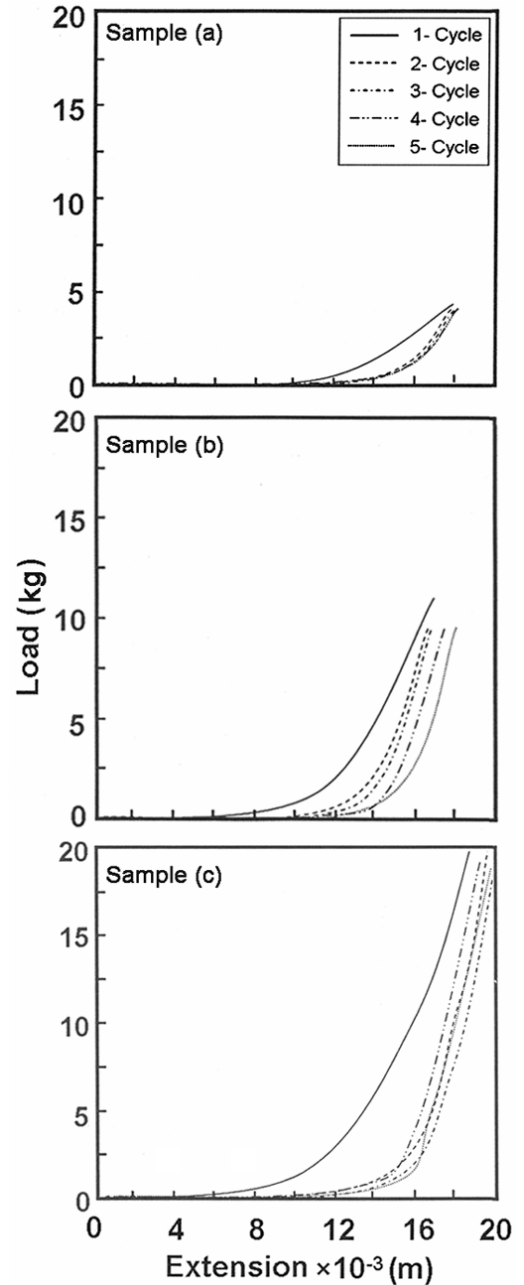


Fig. 7— Load-extension plots of cyclic loading of samples [(a) 2 ply yarn, (b) 4 ply yarn and (c) 8 ply yarn]

3.4 Porosity of Knitted Fabric

The data of knitted fabric thickness and weight are given in the Table 1. The porosity of the knitted fabric is determined by the ratio of void volume to total volume. Porosity value obtained for knitted fabric of 8 ply yarn is 80% which is well within the required range. The area of individual pore was calculated to visualize the openness of the knitted structure. There are two types of pores in knitted fabric, such as in between the two loops and inside the loop. Area of

Table 1—Porosity and area of pores of knitted fabrics

Fabric made with	Thickness cm	Weight gsm	Porosity %	Area of pore, μm^2	
				between the loops	inside the loops
2 ply yarn	0.056	270.0	93	688215	579985
4 ply yarn	0.057	83.75	89	641715	422183
8 ply yarn	0.104	52.50	80	260502	122672

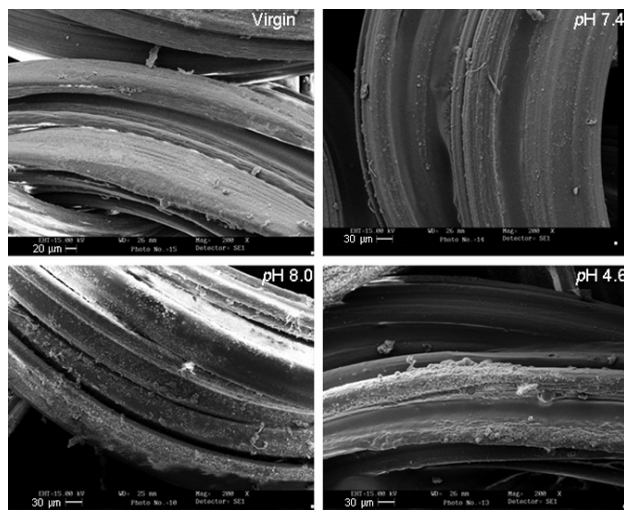


Fig. 8— SEM photographs of knitted fabrics degraded at various pH for period of 20 weeks

these two types of pores is different. The area of pores for different knitted fabrics is given in Table 1. Porosity of the scaffold plays an important role in case of cell culture. It is inessential for the easy passage of nutrients as well as for the three dimensional growth of the tissue culture. The knitted structure is highly porous. The values of porosity obtained for knitted structure are given in Table 1. It is observed that as the number of ply in yarn increases, the porosity decreases. Pore size is also dependent on the number of ply in the yarn. The fabric openness decreases with the increase in the number of ply in the yarn.

3.5 Scanning Electron Microscopy

The knitted PLA scaffold is proposed as the scaffolding material for urinary bladder reconstruction. The urine pH varies from 4.6 to 8.0. Hence, the *in vitro* degradation study was carried out at three different pH (4.6, 7.4 and 8.0) to simulate with the body fluid. The detailed study has been reported elsewhere³⁰. Here, only the surface morphology of the knitted fabric degraded for 20 weeks is reported. It is observed that as the pH

deviates from 7.2, the severity of degradation increases. This is confirmed by images obtained by the scanning electron microscope (Fig. 8). At lower pH, the degradation becomes higher which confirms catalytic effect of hydronium ions on hydrolysis process.

4 Conclusions

The knitted scaffold of dry-jet-wet spun PLA filament is fabricated and evaluated for the performance by various techniques. It is observed that knitted structure properties vary with the number of ply in the yarn. The fabricated scaffold shows considerable higher mechanical properties than actually required for the intended application. Also, the porosity of the fabric is observed to be optimum for the scaffold applications.

Industrial Importance: This work opens up a newer dimension in the field of the development of textile structures for tissue engineering. These materials perform the function of the scaffold which guides the cells for their harvesting into a tissue leading to the subsequent human organ reconstruction. It is possible to design textile knittings with varying porosity and the mechanical strength as well as elasticity which would be useful for the human urinary bladder repair. The area is wide open with enormous possibilities in the field of biotextiles towards textile designing with required physico-chemical features.

Acknowledgement

The authors express their gratitude to Prof. V K Kothari, Department of Textile Technology, Indian Institute of Technology, Delhi, India, for the valuable guidance and support during the study.

References

- 1 Langer R & Vacanti J P, *Science*, 260 (1993) 920.
- 2 Nikolosvski J & Mooney D J, *Biomaterials*, 21 (2000) 2025.
- 3 Huttmacher D W, *Biomaterials*, 21 (2000) 2529.
- 4 Thomson R C, Yaszemski M J & Mikos A G, *Principles of Tissue Engineering*, edited by R Lanza, R Langer and W Chik (R.G. Landes Company, USA), 1997, 263.
- 5 Leong K F, Cheah C M & Chua C K, *Biomaterials*, 24 (2003) 2363.
- 6 Nam Y S & Park T G, *J Biomed Mater Res*, 47 (1999) 8.
- 7 Zhang R & Ma P X, *J Biomed Mater Res*, 44 (1999) 446.
- 8 Yang F, Murugan R, Ramakrishna S, Wang X, Ma Y-X & Wang S, *Biomaterials*, 25 (2004) 1891.
- 9 Freed L E, Marquis J C, Nohria A, Emmanuel J, Mikos A G & Langer R, *J Biomed Mater Res*, 27 (1993) 11.
- 10 Yang Y, Magnay J L, Cooling L & Haj A J E, *Biomaterials*, 23 (2002) 2119.

- 11 Holy C E, Cheng C, Davies J E & Shoichet M S, *Biomaterials*, 22 (2001) 25.
- 12 Fujisato T, Sajiki T, Liu Q & Ikada Y, *Biomaterials*, 17 (1996) 155.
- 13 Heimburg D V, Zachariah S, Heschel I, Kuhling H, Schoof H, Hasemann B & Pallua N, *Biomaterials*, 22 (2001) 429.
- 14 Honda M, Motikawa N, Hata K, Yada T, Morita S, Ueda M & Kimata K, *Biomaterials*, 24 (2003) 3511.
- 15 Mikos A G, Bao Y, Cima L G, Ingber D E, Vacanti J P & Langer R, *J Biomed Mater Res*, 27 (1993) 183.
- 16 Weng J, Wang M & Chen J, *Biomaterials*, 23 (2002) 2623.
- 17 Gupta B, Plummer C, Bisson I, Frey P & Hilborn J, *Biomaterials*, 23 (2002) 863.
- 18 Cui Y L, Qi A D, Liu W G, Wang X H, Wang H, Ma D M & Yao K D, *Biomaterials*, 24 (2003) 3859.
- 19 Freed L E, V-Novakovic G, Biron R J, Eagles D B, Lesnoy D C, Barlow S K & Langer R, *Biotechnology*, 12 (1994) 689.
- 20 Li Y, Ma T, Yang S-T & Kniss D A, *Biomaterials*, 22 (2001) 609.
- 21 Bhattarai S R, Bhattarai N, Yi H K, Hwang P H, Cha D I & Kim H Y, *Biomaterials*, 25 (2004) 2595.
- 22 Wintermantel E, Mayer J, Blum J, Eckert K-L, Lüscher P & Mathey M, *Biomaterials*, 17 (1996) 83.
- 23 Gupta B, Hilborn J, Bisson I, Frey P & Plummer C, *J Appl Polym Sci*, 81 (2001) 2993.
- 24 Bisson I, Kosinski M, Ruault S, Gupta B, Hilborn J, Florian W & Frey P, *Biomaterials*, 23 (2002) 3149.
- 25 Revagade N, Development of bioreceptive poly(ethylene terephthalate) for medical application, M.Tech Thesis, Department of Textile Technology, Indian Institute of Technology, Delhi, India, 2001.
- 26 Gupta B, Revagade N, Anjum N, Atthoff B & Hilborn J, *J Appl Polym Sci*, 100 (2006) 1239.
- 27 Gupta B, Revagade N, Anjum N, Atthoff B & Hilborn J, *J Appl Polym Sci*, 101 (2006) 3774.
- 28 Eenink M J D, Feijen J, Olijslager J, Albers J H M, Rieki J C & Greidanus P J, *J Contr Rel*, 6 (1987) 225.
- 29 Nelson K D, Romero A, Waggoner P, Crow B, Borneman A & Smith G M, *Tissue Engg*, 9 (2003) 1323.
- 30 Gupta B, Revagade N & Hilborn J, *J Appl Polym Sci*, 103 (2007) 2006.