*J. Biomater. Sci. Polymer Edn*, Vol. 16, No. 9, pp. 1179–1192 (2005)

Also available online - www.vsppub.com

*Technical note*

# Characterization of knitted polymeric scaffolds for potential use in ligament tissue engineering

Z. GE [1], J. C. H. GOH [1,2,*], L. WANG [3], E. P. S. TAN [2] and E. H. LEE [1]

[1] *Department of Orthopaedic Surgery, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Republic of Singapore*
[2] *Division of Bioengineering, National University of Singapore, 10 Kent Ridge Crescent, Singapore 119260, Republic of Singapore*
[3] *Institute of Bioengineering and Nanotechnology, 31 Biopolis Way, Singapore 138669, Republic of Singapore*

Received 23 August 2004; accepted 15 February 2005

**Abstract**—Different scaffolds have been designed for ligament tissue engineering. Knitted scaffolds of poly-L-lactic acid (PLLA) yarns and co-polymeric yarns of PLLA and poly(glycolic acid) (PLGA) were characterized in the current study. The knitted scaffolds were immersed in medium for 20 weeks, before mass loss, molecular weight, pH value change in medium were tested; changes in mechanical properties were evaluated at different time points. Results showed that the knitted scaffolds had 44% porosity. There was no significant pH value change during degradation, while there was obvious mass loss at initial 4 week, as well as smooth molecular weight drop of PLLA. PLGA degraded more quickly, while PLLA kept its integrity for at least 20 weeks. Young's modulus increased while tensile strength and strain at break decreased with degradation time; however, all of them could maintain the basic requirements for ACL reconstruction. It showed that the knitted polymeric structures could serve as potential scaffolds for tissue-engineered ligaments.

*Key words*: Poly(lactic acid); ligament; biodegradation; mechanical properties.

## INTRODUCTION

Ligaments are parallel bands of dense connective tissue fibers that mediate normal joint movement and stability. Injury to these structures may result in significant joint dysfunction, which may consequently lead to injury of other tissues and the development of degenerative diseases of the joints. Reconstruction of anterior cruciate ligament (ACL) is the most challenging aspect in all human ligaments. The poor healing capacity of ACL has led orthopedic surgeons to perform ACL

*To whom correspondence should be addressed. Tel.: (65) 6772-4423. Fax: (65) 6774-4082. E-mail: dosgohj@nus.edu.sg

reconstructions rather than repairs, using autografts, allograft, or occasionally artificial ligament replacements. All of them have their own drawbacks, including donor site morbidity, inflammatory reaction, chronic foreign body inflammation and particulate-induced synovitis [1, 2].

Research on biodegradable prostheses for ACL reconstruction has been active with the hope of overcoming these problems. The ideal way is to mimic the normal ACL structures in mechanical properties and gradually transfer mechanical strength to regenerated collagen bundle while degrading [3]. One of main challenges is to fabricate biodegradable scaffolds with mechanical properties matched with normal ACL in terms of strength, compliance, elasticity and durability, not only at initial stage, but also at scaffold degradation and ACL regeneration process. Though bulk structure has been tested for ACL reconstruction [4], most of studies use textile structures. Parallel structure is the simplest way to organize the fibers. However, the lack of interaction between fibers restricts its application. Twisting grafts are morphologically closer copies of the normal ACL and can eventually reduce and fine-tune the peak forces in extension [5, 6]. Due the complexity of the ACL mechanical environment, more complex textile structures have been used in ACL reconstruction, for examples, woven, knitted and braided.

The properties of textile grafts depend on the characteristics of the constituent yarns or fibers and on the geometry of the formed structure. In general, braided scaffolds are usually dimensionally very stable and have high strength and fatigue life, but are less extensible and have limited tissue in-growth due to the low porosity [7]. Braided PLLA ligament augmentation devices have been used for ACL reconstruction in a sheep model [8]. However, the pore size of the braided structures can be regulated by yarn bundle size and braiding angle [9]. Compared with braided fabrics, knitted structures are highly porous, which supports tissue in-growth. It has been reported that the knitted construction of Dacron promotes more in-growth of fibrous tissue than braided Gore-tex prosthesis [10]. Furthermore, knitted PLGA scaffolds have been successfully used in tendon repair [11].

Since collagen accounts for more than 80% of the dry weight of a normal ligament [12], it is reasonable to reconstruct ACL with it. Collagen is the best candidate for reparative cells (fibroblasts) and progenitors cells' adhesion, proliferation and differentiation, as most of material surface modification are based on collagen (or derivatives) coating [13, 14] and Arg-Gly-Asp (RGD) sequence attachment which is abundant in collagen [15]. So far the most matured ACL regeneration is reported from collagen-based ACL regeneration [4, 16]. However, high cost, batch to batch variability, hydrophilicity, complex handling properties, potential immuno-response and disease transmission are existing disadvantages [17]. The extraordinatory mechanical properties and enhanced environmental stability of silk fibers make fibroin (core of silk) suitable for ligament tissue engineering [6, 18]. Though good biocompatibility has been reported, sericin (glue-like protein in silk) remains a concern if not totally removed. So far, there is no *in vivo* study reported, and further experiments are necessary to ensure its safety.

Except for biocompatible and degradable, the materials used in ACL reconstruction should be slowly degradable, as ACL regeneration and subsequent functionality usually needs at least six months [19]. Good cell affinity of materials is critical for regeneration in initial stage, when reparative cell and/or progenitor cell continuously grow in, attach, proliferate and differentiate. At later stage, when materials have been well covered and coated with cells, extracellular matrix and proteins, importance of good cell affinity of materials decreases. Based on these considerations, rapidly degradable PLGA was adopted due to good cell affinity [20], while slowly degradable PLLA could maintain scaffolds' integrity and mechanical properties for a longer time. Both PLLA and PLGA have been well used in many biomedical researches for their safety, ease of processing and good biocompatibility [21]. In the current study, we try to design two-phase degrading structures that meet the basic requirements for ACL reconstruction and characterize them during *in vitro* degradation.

## MATERIALS AND METHODS

### *Fabrication of knitted structure*

PLLA yarns (multifilament, non-braided, 30 filaments, each filament between 15 and 20 $\mu$m in diameter, denier 83) were from Scaffix International (USA). PLGA yarns (ratio of PLA and PGA co-polymer is 10 : 9, 12 filaments per yarn, each filament between 15 and 35 $\mu$m in diameter, denier 50–60) were from Shanghai Tianqing Biomaterial (China). The scaffold was knitted using two PLLA yarns and 1 PLGA yarn with four needles in a knitting machine (SK270, Silver Reed, Suzhou Harisa Machinery, China). The two ends of each 4-cm-long knitted structure were sealed with heat. The scaffolds were washed twice with PBS and then sterilized by immersing in 70% alcohol for 30 min. Subsequently, the scaffolds were immersed in 3 changes of PBS for 15 min before use.

### *Porosity*

The porosity of the knitted scaffolds was estimated by deduction of cross-sectional areas of individual PLLA yarns and PGLA yarns from the gross cross-sectional area of the scaffold. The knitted scaffolds, PLLA yarns and PGLA yarns were fixed under slight tension (approx. 5 N) before the diameter was measured under phase contrast microscope. The following formula was used to calculate the porosity of the scaffolds:

$$\text{Porosity} = 1 - (\text{PLLA area} + \text{PLGA area})/\text{scaffold area},$$

where PLLA area is the sum of all cross-sectional areas of PLLA yarns, PLGA area is the sum of all cross-sectional areas of PLGA yarns and scaffold area is the cross-sectional area of the entire scaffold.

*Molecular weight*

Each sample (1 $\mu$g) was dissolved in 2 ml chloroform (i.e., concentration of sample is around 0.05%) before molecular weights were determined by gel-permeation chromatography (GPC, Waters, USA) at an elution rate of 1 ml/min at 25°C (column: Shodex K-806M, Japan). 100 $\mu$l was analyzed at each time with polystyrene as the standard. Mean values at each time point were compared with one-way ANOVA, LST, $P < 0.05$.

*Mechanical properties of the scaffolds*

The two ends of the scaffolds were sandwiched between pieces of sand paper and secured by superglue (Alteco 110, Alteco Chemical, Singapore) to prevent slippage of the scaffolds between the pneumatic grips used in the tensile test. The scaffolds had a gauge length of 20 mm. The tensile properties were measured with the Instron 5848 Micro Tester at a constant strain rate of 0.8%/s. The viscoelastic properties were studied by carrying out relaxation and creep tests. In both viscoelastic tests, a pre-load of 0.2 N was first applied before pre-conditioning the scaffolds for 10 cycles at a frequency of 0.1 Hz and amplitude of 2% gauge length. The scaffolds were then stretched to 2.5% strain for relaxation tests, 1.5 N load for creep test and held at the respective strain and load values for 900 s. The strain and load levels selected for the viscoelastic tests correspond to the range of strain experienced *in vivo* [22]. The values of stress were obtained by dividing the load by the cross-sectional area of the scaffold. The first linear region (i.e., at lower strain levels) was used for determination of modulus. The normalized stress relaxation rate is obtained by finding the slope of the normalized stress *vs*. $\ln(t)$ strain plot. The normalized stress is obtained by dividing the stress values by the initial stress immediately after the initial 2.5% strain has been applied (i.e., stress at time zero). The logarithmic operation on the time axis transformed the inherently nonlinear relation in load decay *vs*. time into a linear relation such that a simple linear regression could be used to determine the rates of relaxation [23]. The cross-sectional area of the scaffold was taken to be the sum of cross-sectional areas of the individual filaments measured under an optical microscope. The strain values were obtained by dividing the elongation by the gauge length of the specimen.

In vitro *degradation*

Knitted scaffolds were immersed in 1 ml of culture medium in 15-ml centrifuge tubes. The systems were incubated at 37°C, 5% $CO_2$, while medium was changed weekly. The pH values from three centrifuge tubes were measured as a group, using a pH meter (Coring PH meter 430). Eight of nine replicates were used at each time point (3 for mass loss followed by GPC and 5 for mechanic tests). The scaffolds were immersed for 4, 8, 12, 16 and 20 weeks, and then 3 replicates were lyophilized, weighed and analyzed using GPC while 5 replicates were used in mechanical tests.

## RESULTS AND DISCUSSION

The acquired knitted scaffolds of PLLA and PLGA were blue porous, cord-like structures with in a gross diameter of about 1 mm (Fig. 1).

### *Porosity*

The porosity of the knitted scaffolds was 44% (Table 1). It is important that tissue engineering scaffolds have sufficient porosity to accommodate tissue regeneration and nutrient exchange following implantation. It is advantageous to have a polymer construct with a large surface area-to-volume ratio [24], as such a construct provides a conduit for uniform cell delivery and development of high cell density. One of the

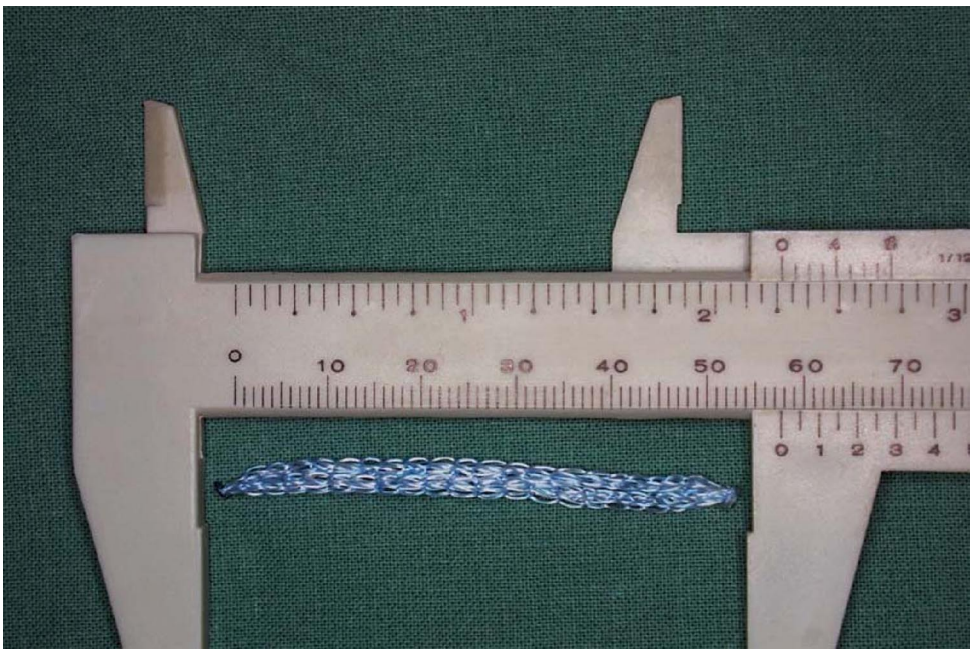


**Figure 1.** Knitted structure of PLLA and PLGA. This figure is published in colour on http://www.ingenta.com

**Table 1.**
Porosity of scaffolds

| | Diameter ($\mu$m) | | | | | Average diameter ($\mu$m) | Area (mm$^2$) | No. of yarns | Total area (mm$^2$) | % |
|---|---|---|---|---|---|---|---|---|---|---|
| | Sample 1 | Sample 2 | Sample 3 | Sample 4 | Sample 5 | | | | | |
| PLLA | 166.3 | 178.2 | 173.2 | 178.2 | 187.0 | 176.6 | 0.0245 | 16 | 0.392 | 45 |
| PLGA | 126.9 | 119.7 | 122.0 | 124.6 | 124.4 | 123.5 | 0.012 | 8 | 0.096 | 11 |
| Scaffold | 1241.6 | 1091.5 | 1041.0 | 963.8 | 919.7 | 1051.5 | 0.87 | 24 | 0.87 | 100 |

criteria for an ideal scaffold was that the porosity should be at least 90% in order to provide a high surface area for cell–polymer interactions, sufficient space for extracellular matrix (ECM) regeneration and minimal diffusion constraints during *in vitro* culture. However, with regards to *in vivo* biodegradation, scaffolds with low porosity (relatively with low surface to volume ratio) can possibly be of more advantage, as the slow degradation will keep the integrity of the original constructs longer, as hydrolysis of both PLLA and PLGA starts from surface [25]. In order to comply with these two considerations, the porosity of 44% (current knitted structures) was used.

### In vitro *degradation*

During the 20-week immersion period, the pH value of the immersing medium remained at 7.4, but dropped slightly below 7 at week 3 (Fig. 2). In general, the trace amount of acidic degradation products, mainly polymers of lactic acids and glycolic acids, will not interrupt the internal human environment by the abrupt drop in pH value, as they are rapidly cleared [26]. There should be no significant decline in pH value *in vivo* at week 3, as the degradation products are continuously cleared, unlike our *in vitro* experiments. The drop of pH value at week 3 was possibly due to quick degradation of PLGA.

The mass of the knitted structures decreased with time as predicted following immersion (Fig. 3). Two statistical significances have been observed, between initial weight and all remaining groups, as well as between 4 and 20 weeks (one-way ANOVA, LSD, $P < 0.05$). The first significant difference could possibly be attributed to relatively quick degradation of PLGA, which was justified by loss of blue color of the scaffolds at the initial stage (Fig. 4) and the pH value drop at week 3. The second significant difference is also reasonable. As mass loss of the scaffolds started gradually, it became apparent at 20 weeks. PLGA yarns degraded faster than PLLA yarns, as the blue PLGA yarns were not visible at 8 weeks.

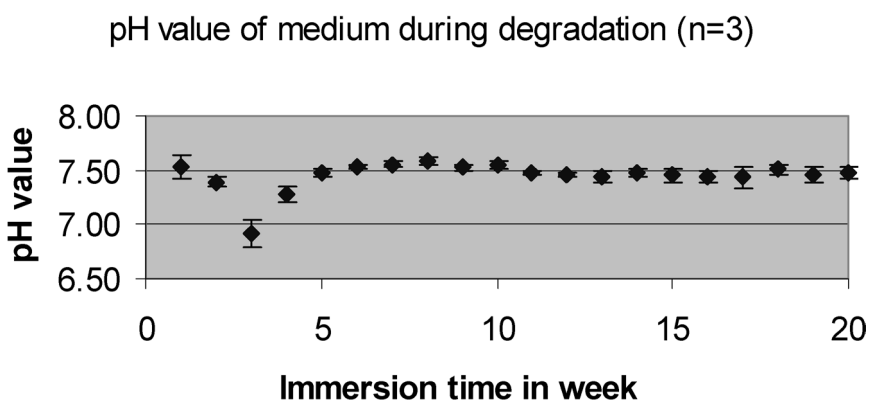


**Figure 2.** Change of pH value of immersion medium.

The molecular weights of PLLA at different immersion durations were measured. As the rapidly degrading PLGA was essential to promote potential tissue in-growth at the initial stage of implantation, as well as to provide space for regeneration at later stage, its molecular weight was not examined. Furthermore, for PLLA, being the backbone of our scaffold, its degradation and deterioration rate were critical. PLGA did not dissolve in chloroform, which was used to dissolve PLLA for the GPC test. Hence the presence of PLGA would not interfere with the analysis of the molecular weight of PLLA. There was a descending trend in molecular weights of PLLA with time (Fig. 5). The drop in molecular weight usually started earlier than mass loss [3]. However, there was no statistical significance until week 12, possibly due to small differences between different groups. The further decline in molecular weight at 16 and 20 week attributed to the statistical significance between them and other groups (one-way ANOVA, LST, $P < 0.05$).

The main parameters which influence the polymer biodegradability are polymer crystallinity, hydrophilicity, composition and form of the product. PLLA degrades by backbone breakage caused by water penetration. This can be rephrased as 'the hydrophilicity of the polymer is increased so is its biodegradability', because the solvent in the biological media is basically water with a high salt content [25]. Slow degradation of semi-crystalline PLLA due to relatively hydrophobic property attributed to its usage in the potential ligament tissue-engineered structures.

For tissue engineering, the ability to control the degradability of the biomaterials is critical in order to achieve its objective. The ideal rate of degradation should not exceed the rate of tissue regeneration, especially with regards to mechanical strength. In spite of the large number of investigations dealing with PLA, PGA and their co-polymers, they still degrade in varying rate when implanted at various locations and under different mechanical stimulus. Recent data obtained from *in vitro* ageing experiments under physiological conditions show that initial and subsequent morphology changes are very important factors in the determination of

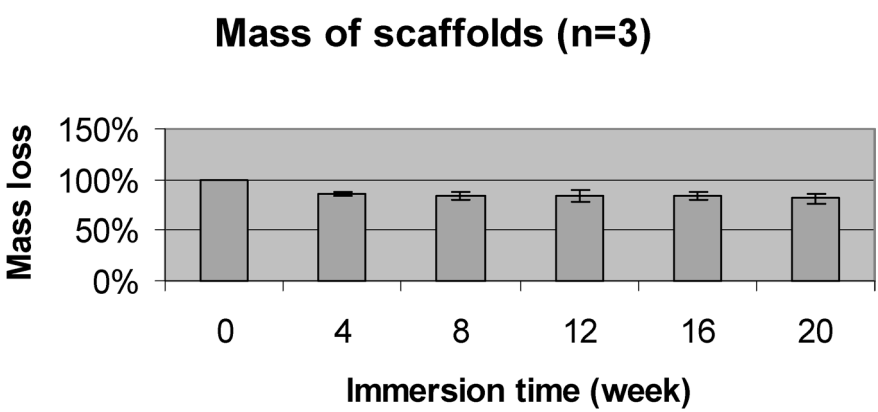


**Figure 3.** Mass loss of knitted structure during degradation.

the degradation behaviors of these polymers [27]. Hence, this current study on degradation of knitted structures is meaningful.

The *in vitro* degradation experiment design described in this paper was based on ISO standard 13781, 'Poly(L-lactide) resins and fabricated forms for surgical implants — *in vitro* degradation testing', with small revisions. Cell-culture medium was used instead of standard soaking solution, as current structures should possibly

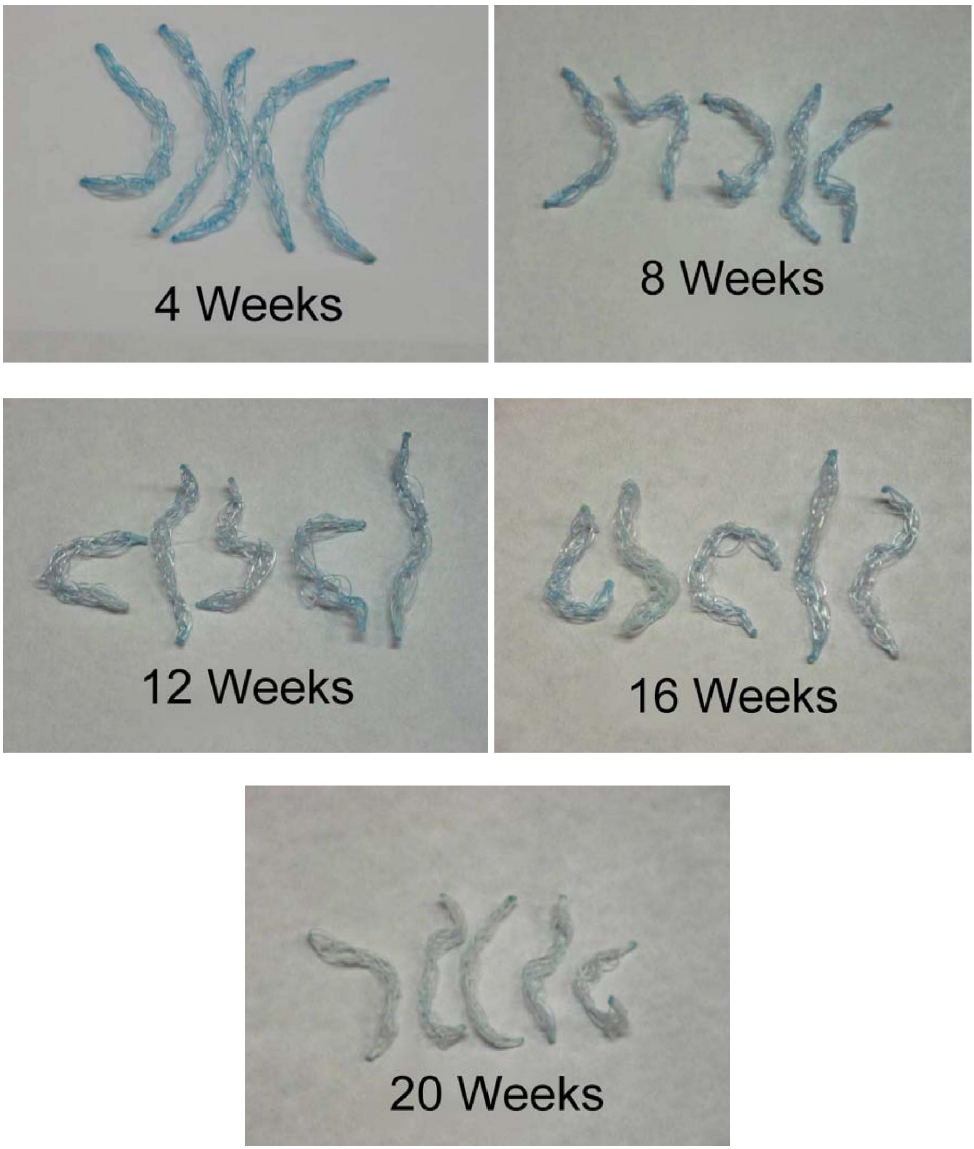


**Figure 4.** Macroscopic changes of scaffolds in immersion. This figure is published in colour on http://www.ingenta.com

be immersed in it during future *in vitro* and *in vivo* cell-loading procedures and bioreactor incubations.

*Mechanical test of scaffolds*

*Tensile properties.* The stress–strain behavior of the scaffold displays the characteristic toe, linear, yield and failure regions that are typically found in stress-strain curves of tendons and ligaments [22], as shown in Fig. 6. The modulus decreased at 4 weeks and then increased with degradation time (Fig. 7a) and the ultimate tensile strength and strain decreased with degradation time (Fig. 7b and 7c). This trend has also been observed in other *in vitro* degradation studies as well [28, 29], while the initial decrease at 4 week could be attributed to quick loss of PLGA

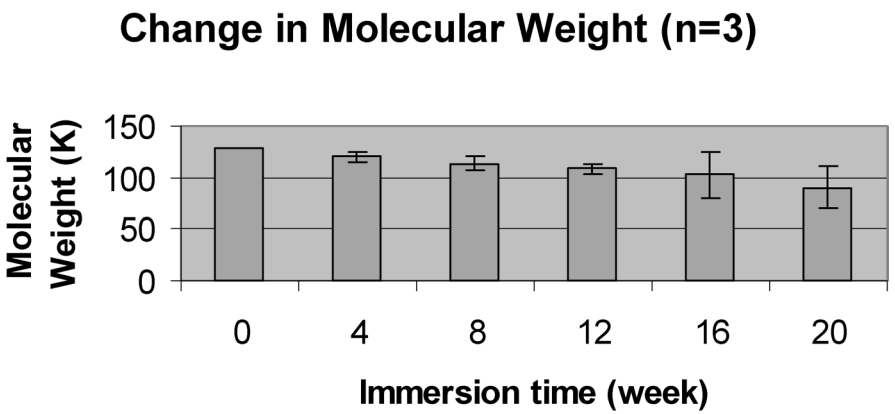


**Figure 5.** Changes in molecular weight at different immersion durations.

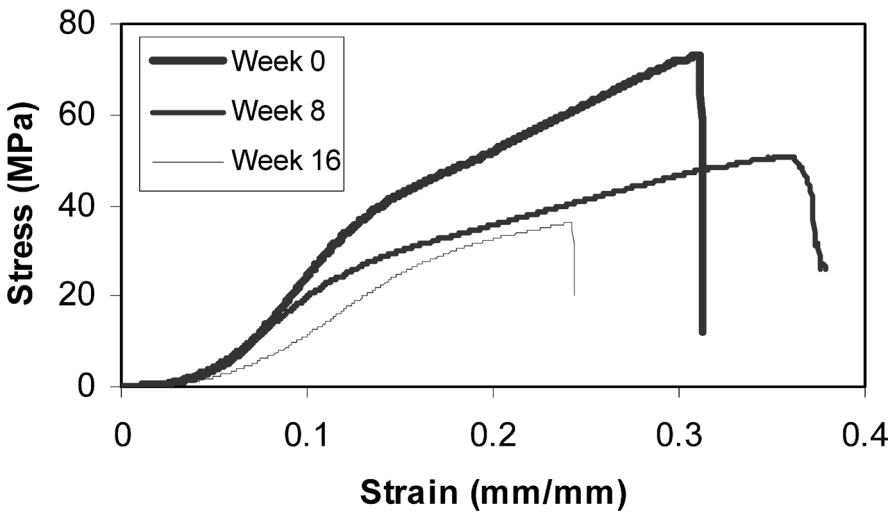


**Figure 6.** Stress–strain plots of PLLA/PLGA scaffolds at different duration of immersion in the medium.

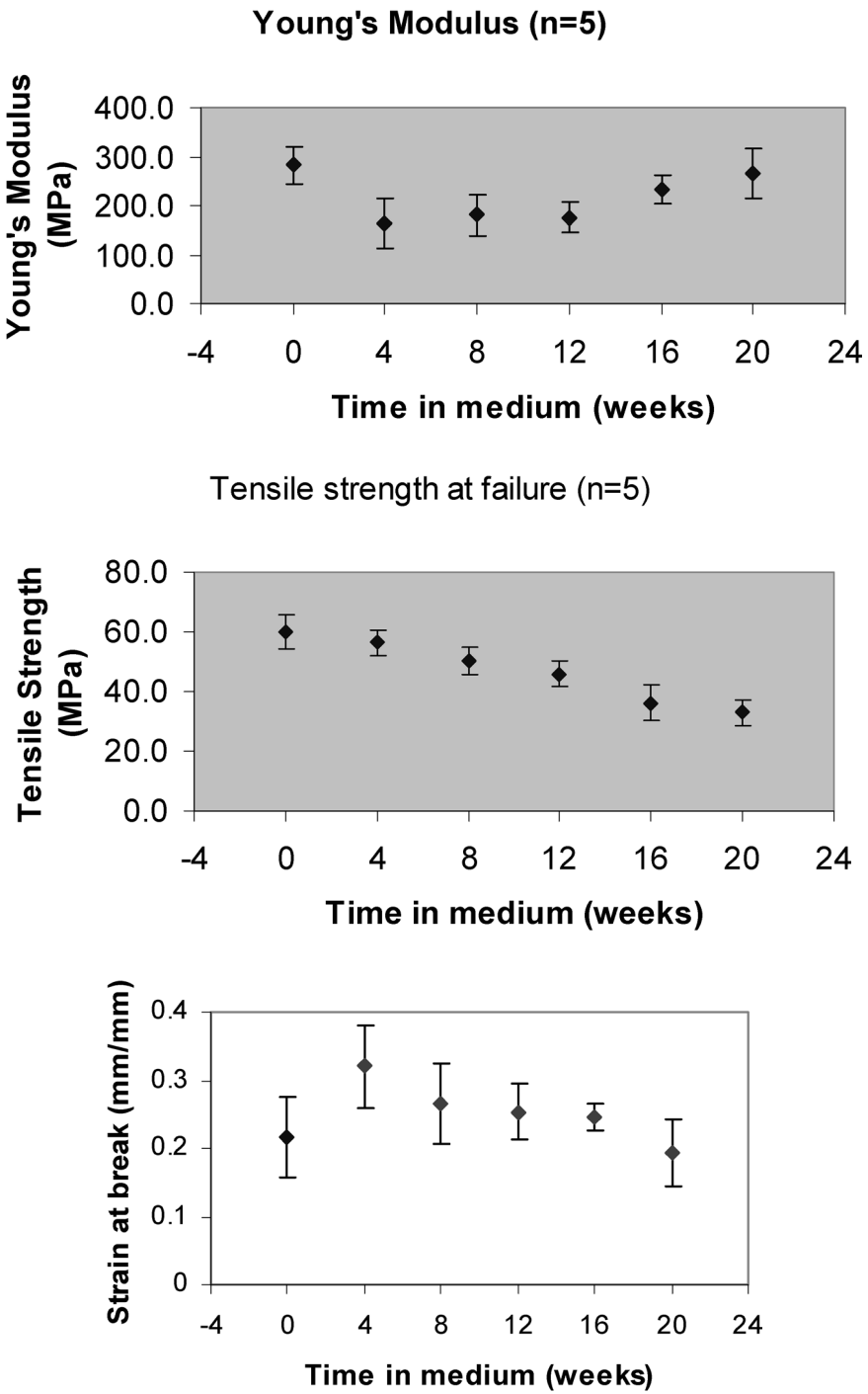


**Figure 7.** Tensile properties of PLLA/PLGA scaffold as a function of *in vitro* degradation time in the medium. Top: Young's modules; middle: tensile strength at failure; bottom: tensile strain at break.

yarns. The increase in crystallinity during degradation at the amorphous regions are first hydrolyzed which resulted in a higher Young's modulus with degradation time [29, 30]. The increase in mass loss and simultaneously decline in molecular weight, resulted in more defects within the scaffold and thus the reduced ultimate tensile strength and strain.

The initial reduction in Young's modulus and increase in ultimate strain between week 0 and week 4 indicated that the scaffold became less stiff and more ductile, possibly due to degradation of PLGA fibers which are stiffer and less ductile than PLLA. This trend is contrary to that observed at subsequent degradation time points. The combined effect of immersion at an elevated temperature of 37°C in the medium could have resulted in some structural changes of the scaffold before the effects of hydrolysis became significant.

The Young's modulus of the scaffold at various durations of immersion in the medium ranges from 146% to 238% of the value reported for human ACL [31]. In general, the Young's modulus value for human anterior cruciate ligament is 111 MPa, ultimate tensile strength is at least 38 MPa [31], while ultimate mechanical properties of ligaments generally increase during development and then diminish with aging [32]. The ultimate tensile strength before 12 weeks of immersion in medium complies with the minimum reported strength of 38 MPa, while ultimate tensile strength at 16 weeks and 20 weeks account for 95% and 87%, respectively. It is possible that the new ligament tissue regenerated within 12–16 weeks is sufficient to withstand the *in vivo* stresses as the tensile strength of the scaffold is reduced significantly after 16 weeks. Any compliance mismatch (difference in Young's modulus) between tissue and scaffold could result in failure of the implant [23, 33]. However, the current structures will undergo many procedures, such as cell loading, ECM laying down and subsequent cross-linking, mechanical stimulus in bioreactors, biomembrane wrap, etc. All these procedures would possibly alter the final Young's modulus and ultimate tensile strength. As ultimate tensile strength and Young's modulus of the current constructs were similar with human ACL, they could be a good candidate of scaffolds used in ligament tissue engineering.

The strains at break fell between 19.4 and 32% during the 20-week immersion. There is much controversy about ACL's strain at break, mostly due to the difference of mechanical procedures and standard of failure. Further loading beyond the toe region produces a nearly linear curve when fibers lose their crimp and become parallel. The upper strain limit of this linear region is 2–5% and then collagen fiber failure begins at 7–8% strain [34]. The maximum strain that a ligament can endure before failure is between 12 and 15% [22]. However, a further 20–40% apparent linear region was reported when the properties of whole ligaments were examined [35, 36]. There were several possible artifacts in the testing technique. First, the initial failure of a small number of collagen fibers was not detected, while not affecting the load carrying ability of the ligament as a whole. Second, the measurement of the travel between the grips provides an overestimation of the true tissue strain without high-speed video recording. Third, pre-conditioning to

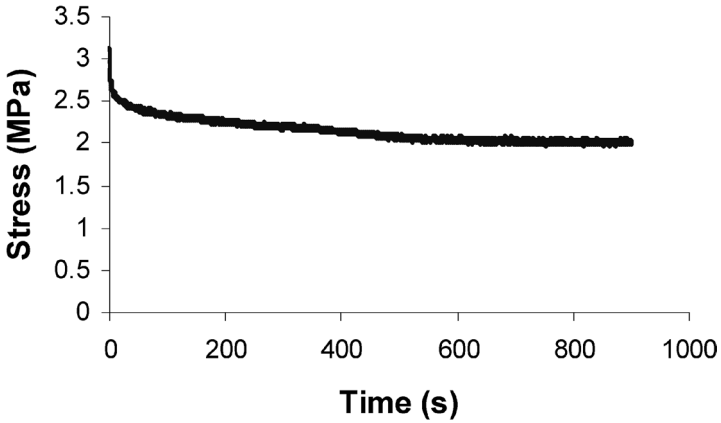


**Figure 8.** Stress relaxation curve of PLLA/PLGA scaffold with initial strain of 2.5%.

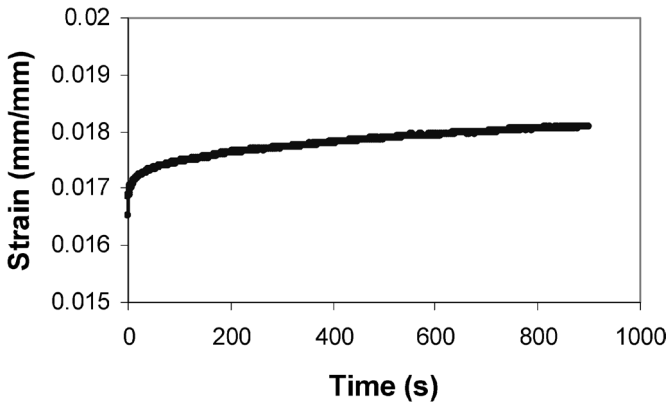


**Figure 9.** Creep curve of PLLA/PLGA scaffold with initial load of 1.5 N.

align the individual fibers and different strain rates used in different experiments also contributed to the differences [36, 37]. Current results could almost match that of normal ACL when ligaments were tested as a whole component.

*Viscoelastic properties.* The stress needed to sustain a constant elongation decreased with time (Fig. 8) and the strain required to sustain a constant load increased with time (Fig. 9), indicative of a viscoelastic behavior under mechanical loading, identical to normal ACLs [36–38]. Normalized stress relaxation rate of the scaffold obtained from the relaxation test was $-0.041 \pm 0.009/\ln(s)$. Normalized strain creep rate of the scaffold obtained from creep test was $0.013 \pm 0.001/\ln(s)$. The purpose of using normalized stress and strain is to eliminate the effect of initial applied strain and stress in relaxation and creep tests, respectively.

The normalized stress relaxation rate obtained ($-0.041/\ln(s)$) is comparable to that obtained by Donahue *et al*. [39] in their study of anterior cruciate ligament (ACL) grafts made from bovine digital extensor ($-0.038/\ln(s)$) and human ham-

string tendons ($-0.036/\ln(s)$). The normalized strain creep rate ($0.013/\ln(s)$), however, is lower than that obtained although it is of the same order of magnitude. Normalized strain creep rate for bovine digital extensor is $0.029/\ln(s)$ and that for human hamstring tendons is $0.025/\ln(s)$. The difference is probably due to the different testing methodologies and the fact that different materials are used (i.e., biological samples *vs*. polymeric scaffold). However, the proximity of the values indicates that the PLLA/PLGA scaffold is a viable implant for ACL regeneration in terms of viscoelastic behavior.

The past 10 years have seen huge efforts to develop biomaterials that can be considered as 'ideal' scaffolds for cell growth however few have reached clinical efficacy. Regardless of the source or type of biomaterials, they have to be biocompatible and mechanically compatible with native tissues to fulfill their desired role. Few of them have been reported suitable for ligament tissue engineering, especially when both appropriate mechanical properties and general requirements for tissue engineering are required [40].

## CONCLUSIONS

The knitted PLLA/PLGA structures developed in this study can fulfill most of the requirements for prospective candidates for ligament tissue engineering with regards to porosity, degradation rate and mechanical properties. However, more research has to be carried out to improve and revise the mechanical properties of current knitted scaffolds before they can finally been used in clinical practices.

*Acknowledgements*

This research is supported by a National Medical Research Council grant (R-175-000-037-213). We would like to appreciate the kind assistance of Mr. Lau Soo Yong for mechanical tests.

## REFERENCES

1. R. H. Miller, in: *Campbell's Operative Orthopaedics*, S. T. Canale (Ed.), p. 2274. Mosby, St. Louis, MO (2003).
2. J. A. Koski, C. Ibarra and S. A. Rodeo, *Orthoped. Clin. North. Am.* **31**, 437 (2000).
3. D. W. Hutmacher, *Biomaterials* **21**, 2529 (2000).
4. D. W. Jackson, T. M. Simon, W. Lowery and E. Gendler, *Am. J. Sports Med.* **24**, 405 (1996).
5. M. P. Arnold, L. Blankevoort, A. ten Ham, N. Verdonschot and A. van Kampen, *J. Orthoped. Res.* **22**, 963 (2004).
6. G. H. Altman, R. L. Horan, H. H. Lu, J. Moreau, I. Martin, J. C. Richmond and D. L. Kaplan, *Biomaterials* **23**, 4131 (2002).
7. G. Vunjak-Novakovic, G. Altman, R. Horan and D. L. Kaplan, *Annu. Rev. Biomed. Eng.* **6**, 1 (2004).
8. O. Laitinen, T. Pohjonen, P. Törmälä, K. Saarelainen, J. Vasenius, P. Rokkanen and S. Vainionpää, *Arch. Orthoped. Trauma Surg.* **112**, 270 (1993).

9. J. A. Cooper, H. H. Lu, F. K. Ko, J. W. Freeman and C. T. Laurencin, *Biomaterials* **26**, 1523 (2005).
10. G. R. Barrett, L. L. Line, Jr., W. R. Shelton, J. O. Manning and R. Phelps, *Am. J. Sports Med.* **21**, 367 (1993).
11. H. W. Ouyang, J. C. Goh, A. Thambyah, S. H. Teoh and E. H. Lee, *Tissue Eng.* **9**, 431 (2003).
12. R. C. Haut, in: *The Anterior Cruciate Ligament: Current an Future Concepts*, D. W. Jackson, S. P. Arnoczky, C. B. Frank, S. L.-Y. Woo and T. M. Simon (Eds), p. 63. Raven Press, New York, NY (1993).
13. E. Domotor, I. Sipos, A. Kittel, N. J. Abbott and V. Dam-Vizi, *Neurochem. Int.* **33**, 473 (1998).
14. A. Hautanen, J. Gailit, D. M. Mann and E. Ruoslahti, *J. Biol. Chem.* **264**, 1437 (1989).
15. S. P. Massia and J. A. Hubbell, *Ann. N.Y. Acad. Sci.* **589**, 261 (1990).
16. M. G. Dunn, A. J. Tria, P. Kato, J. R. Bechler, R. S. Ochner, J. P. Zawadsky and F. H. Silver, *Am. J. Sports Med.* **20**, 507 (1992).
17. C. H. Lee, A. Singla and Y. Lee, *Int. J. Pharm.* **221**, 1 (2001).
18. G. H. Altman, F. Diaz, C. Jakuba, T. Calabro, R. L. Horan, J. Chen, H. Lu, J. Richmond and D. L. Kaplan. *Biomaterials* **24**, 401 (2003).
19. D. Amiel, E. Billings and W. H. Akeson, in: *Knee Ligaments: Structure, Function, Unjury, and Repair*, D. Daniel, W. Akeson and J. O'Connor (Eds), p. 77. Raven Press, New York (1990).
20. H. W. Ouyang, J. Goh and E. H. Lee, *Mater. Sci. Eng. C* **20**, 63 (2002).
21. B. E. Chaignaud, R. S. Langer and J. P. Vacanti, in: *Synthetic Biodegradable Polymer Scaffolds*, A. Atala and D. Mooney (Eds), p. 1. Birkhauser, Boston, MA (1997).
22. R. B. Martin, D. B. Burr and N. A. Sharkey, in: *Skeletal Tissue Mechanics*, p. 309. Springer-Verlag, New York, NY (1998).
23. H. J. Salacinski, S. Goldner, A. Giudiceandrea, G. Hamilton, A. M. Seifalian, A. Edwards and R. J. Carson, *J. Biomater. Appl.* **15**, 241 (2001).
24. B. S. Kim and D. J. Mooney, *Trends Biotechnol.* **16**, 115 (1996).
25. V. Hasirci, in: *Biomaterials and Bioengineering Handbook*, D. C. Wise (Ed.), p. 141. Marcel Dekker, New York, NY (2000).
26. M. Miyaguchi, A. Kobayashi, Y. Kadoya, H. Ohashi, Y. Yamano and K. Takaoka, *Osteoarthr. Cartil.* **11**, 252 (2003).
27. M. Vert, S. Li and H. Garreau, *J. Control. Rel.* **16**, 15 (1991).
28. M. S. Widmer, P. K. Gupta, L. Lu, R. K. Meszlenyi, G. R. Evans, K. Brandt, T. Savel, A. Gurlek, C. W. Patrick, Jr. and A. G. Mikos, *Biomaterials* **19**, 1945 (1998).
29. X. Yuan, A. F. T. Mak and K. Yao, *Polymer Degrad. Stabil.* **79**, 45 (2003).
30. G. Perego, G. Demenico and C. Bastilio, *J. Appl. Polym. Sci.* **59**, 37 (1996).
31. F. R. Noyes and E. S. Grood, *J. Bone Joint Surg. Am.* **58**, 1074 (1976).
32. S. L.-Y. Woo, J. M. Hollis and D. J. Adams, *Am. J. Sports Med.* **19**, 217 (1991).
33. S. E. Greenwald and C. L. Berry, *J. Pathol.* **190**, 292 (2000).
34. J. S. Shah, M. I. Jayson and W. G. Hampson, *Ann. Rheum. Dis.* **36**, 139 (1977).
35. F. R. Noyes, J. L. DeLucas and P. J. Torvik, *J. Bone Joint Surg. Am.* **56**, 236 (1974).
36. F. R. Noyes, C. S. Keller, E. S. Grood and D. L. Butler, *Med. Sci. Sports Exerc.* **16**, 427 (1984).
37. F. R. Noyes, D. L. Butler, E. S. Grood, R. F. Zernicke and M. S. Hefzy, *J. Bone Joint Surg. Am.* **66**, 344 (1984).
38. R. S. Boorman, G. M. Thornton, N. G. Shrive and C. B. Frank, *Acta Orthoped. Scand.* **73**, 568 (2002).
39. T. L. H. Donahue, C. Gregersen, M. L. Hull and S. M. Howell, *Trans. ASME* **123**, 162 (2001).
40. M. S. Chapekar, *J. Biomed. Mater. Res.* **53**, 617 (2000).