Journal of Applied Polymer Science

Vitamin E Acetate Addition to Poly(D,L)Lactic Acid Modifies Its Mechanical Behavior Without Affecting Biocompatibility

Pamela Pittarella,[1] Diego Antonioli,[2] Manuela Rizzi,[1] Michele Laus,[2] Filippo Renò[1]

[1]Innovative Research Laboratory for Wound Healing, Health Sciences Department, University of Eastern Piedmont "A. Avogadro," Via Solaroli 17, 28100 Novara, Italy

[2]Department of Sciences and Technological Innovation, University of Eastern Piedmont "A. Avogadro," V. Teresa Michel 11, 15121 Alessandria, Italy

Correspondence to: F. Renò (E-mail: filippo.reno@med.unipmn.it)

ABSTRACT: Mechanical properties of poly(D,L)lactic acid films enriched with Vitamin E and Vitamin E Acetate (5–40% w/w) were investigated. The addition of both formulations resulted in increased polymer Young's modulus and tensile strength. Human foreskin fibroblasts and murine pre-osteoblasts were used to assess the biocompatibility of polymers. Pre-osteoblasts adhesion and proliferation were strongly decreased by Vitamin E, whereas Vitamin E Acetate did not alter cell proliferation. Collagen deposition was lower onto Vitamin E blended polymers than onto native and Vitamin E Acetate blended ones. Fibroblasts adhesion and proliferation were increased by both Vitamin E and Vitamin E Acetate addition. © 2013 Wiley Periodicals, Inc. J. Appl. Polym. Sci. **2014**, *131*, 39970.

KEYWORDS: biocompatibility; biomaterials; blends; films; mechanical properties

Received 9 August 2013; accepted 12 September 2013
DOI: 10.1002/app.39970

INTRODUCTION

Biodegradable polymers are widely used in the medical field, both as drug delivery devices and scaffold for tissue engineering. Their wide use in this field is because of the fact that after their implantation they do not require second surgery for removal, as they can degrade and gradually be absorbed or eliminated by the body. [1,2]

In particular, polylactic acid (PLA) is a biocompatible aliphatic polyester that can degrade into nontoxic compounds, specifically lactic acid, which is metabolized through the tricarboxylic acid cycle and excreted as carbon dioxide and water. [3,4] In fact, PLA was approved by the US Food and Drug Administration as far back as in the 1970s and is primarily used for biomedical applications such as absorbable sutures, stents, drug delivery systems, bone fixation devices, and tissue engineering scaffolds. [4–7]

As lactide is an enantiomeric compound, the general name PLA refers to three possible isoforms, namely poly (L-lactide) (PLLA), poly (D-lactide) (PDLA), and poly (D,L-lactide) (PDLLA), which are chemically identical, but differ in their crystallinity. PLLA is a semicrystalline polymer characterized by high tensile strength, low elongation, and consequently high Young's modulus value, making it suitable for orthopedic applications and sutures. On the contrary, P(D,L)LA is an amorphous polymer exhibiting lower tensile strength, higher elongation, and lower modulus, and is then less applicable than crystalline polymers for load-bearing applications. Moreover, P(D,L)LA, because of its amorphous structure, can degrade quickly; in fact, the degradation time of P(D,L)LA is about 12–16 month, compared to 3–5 years required by PLLA to be completely degraded. Moreover, it is an interesting drug delivery system, when it is essential to have a homogeneous dispersion of the drug within a monophasic matrix. [3,8,9] Although P(D,L)LA mechanical properties are not adequate to fix the majority of bone fracture, it has been extensively investigated for biomedical coating for orthopedic implants because of its good osteoconductive potential, high mechanical adherence to substrates and excellent biocompatibility *in vivo*.[10–12]

Vitamin E (α-tocopherol, Vit. E) [Figure 1(A)] is a natural biological anti-inflammatory and antioxidant agent protecting cells from the damaging effects of free radicals by preventing peroxides accumulation, and can potentially be used to prevent diseases associated with oxidative stress.[13,14] Moreover, Vit.E is also believed to act via antioxidant-independent mechanism, directly affecting blood coagulation, connective tissue growth, inflammation, and cell proliferation.[15,16] Furthermore, several studies indicate that Vit. E has a positive effect on bone tissue, preventing bone loss, maintaining bone mineral density, and improving its mechanical properties. [17–19]

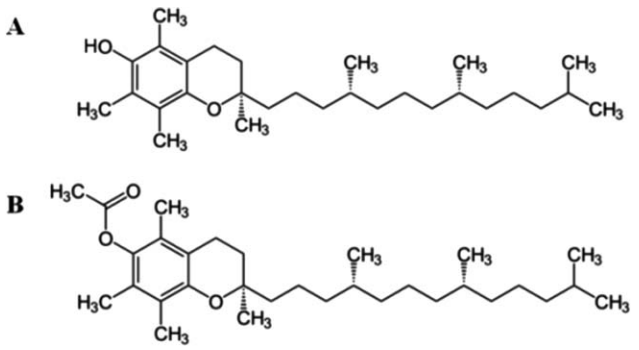

Figure 1. Chemical structures: chemical structure of (A) Vit. E and (B) Vit. E Ac.

Vitamin E acetate (Vit. E Ac, tocopheryl acetate) [Figure 1(B)], is the artificial esterified form of Vit. E, commonly used as an alternative to tocopherol itself because its phenolic hydroxyl group is blocked resulting in a less acidic product. It is believed that this molecule, when absorbed at skin level, undergoes hydrolysis, regenerating alpha tocopherol.[20]

In our laboratory P(D,L)LA films enriched with Vit. E, showing increased wettability and haemocompatibility were produced.[21,22]

In this article, the effects of different concentrations (5–40% w/w) of Vit. E and Vit. E Ac to P(D,L)LA films on the polymer mechanical properties have been investigated, along with the cellular behavior of human foreskin fibroblast (HFF) cells and MC3T3 murine pre-osteoblast cells seeded onto the control and Vit. E and Vit. E Ac enriched P(D,L)LA (10% w/w) films.

EXPERIMENTAL

Preparation of P(D,L)LA Films

P(D,L)LA (average molecular mass 75–120 kDa), vitamin E (α-tocopherol) and Vit. E Ac were purchased from Sigma-Aldrich (Milwaukee, WI, USA). P(D,L)LA films were prepared by casting 0.05 g/mL P(D,L)LA solution in chloroform with or without Vit. E and Vit. E Ac in 150-mm glass dishes. After 5 min shaking 5%, 10%, 20%, and 40% (w/w) Vit. E or Vit. E Ac, both diluited 1 : 1 in chloroform, were added to P(D,L)LA solution and the solvent was evaporated at room temperature for 24 hours under vacuum in the dark. Film sheets (approx. 1-mm thick) were then cut under sterile conditions into square samples (1 cm^2) and stored at 4°C for no more than 1 week.

Thermal and Mechanical Characterization

Differential scanning calorimetry (DSC) was carried out using a Mettler-Toledo DSC 821 apparatus. Samples of about 5 mg were employed. The instrument was calibrated with high purity standards. Dry nitrogen was used as purge gas. All film samples were preconditioned in a vacuum oven set at 50°C for 24 h. The thermal cycle consists in a first heating at 20°C/min from -65°C to 200°C, then to cancel the thermal history, first cooling at −10°C/min to −65°C and second heating at 10°C/min to 200°C. The mechanical tests were performed using a dynamical-mechanical analyzer DMTAV (Rheometer Scientific). All tests were carried out at a temperature of 25°C using the rectangular tension geometry on specimen machined into bars with size of 20 × 5 × 0.01 mm with a gauge length of 10 mm. The stress–strain mechanical analysis was performed at a shear rate of 0.01 s^{-1} with a preload force of 0.01 N. Five measurements were carried out on different specimens for each composite sample.[23,24]

Cell Culture

HFF, established from normal human foreskins pooled from three to five donors, obtained from ATCC (Manassas, VA, USA), was a kind gift from Dr. M. Landini, Microbiology Laboratory, University of Eastern Piedmont "A. Avogadro". HFF cells were grown in Dulbecco's Modified Eagle's Medium (DMEM, Euroclone, Milan, Italy) supplemented with 10% foetal bovine serum (FBS), penicillin (100 U/mL), streptomycin (100 mg/mL) and L-glutamine (2 m*M*) (Euroclone, Milan, Italy) in a humidified atmosphere containing 5% CO_2 at 37°C.

Murine pre-osteoblasts MC-3T3 E1 (ATCC CCL-240) were grown in Iscove's Modified Dulbecco's Medium (IMDM) medium (Euroclone, Milan, Italy) supplemented with 10% heat inactivated FBS, penicillin (100 U/mL), streptomycin (100 mg/mL) and L-glutamine (2 m*M*) (Euroclone, Milan, Italy) in a humidified atmosphere containing 5% CO_2 at 37°C.

Cell Adhesion and Proliferation

Cell adhesion and proliferation onto native, unmodified and modified P(D,L)LA films was evaluated by dropping 1 × 10^4 cells/50 μL onto control and 10% Vit. E and Vit. E Ac P(D,L)LA samples (1 cm^2). Cell adhesion was evaluated after 6 hours incubation, whereas cell proliferation has been evaluated after 72 hours of incubation by fluorescence microscopy. At the end of each time point, cells were fixed in 3.7% formaldehyde-3% sucrose solution in PBS (pH = 7.4) and stained with 1% acridine orange aqueous solution for 5 min in the dark. Stained cells were counted in 10 different fields per sample at 10× magnification using a fluorescence microscope (Leica DM500). Scoring was performed by three separated observes, blind to P(D,L)LA type observed, using Particule software and expressed as total adherent cell number/mm^2 ± standard deviation (S.D.).

Determination of Collagen Production

Sirius Red staining was performed to evaluate collagen production. It is a strong anionic dye with sulfonic acid groups that interact with the basic groups of collagen, resulting in red staining of collagen fibers. [25] Sirius red powder (Sigma Aldrich, St. Louis, MO, USA) was dissolved in saturate aqueous picric acid at a 100 mg/100 mL concentration. P(D,L)LA fixed samples were air dried before adding the dye. Cells were stained for 1 hour under mild shaking. The solution was then removed and the stained cells were extensively washed with 0.01*N* hydrochloric acid. The stained material was dissolved in 0.01*N* sodium hydroxide for 30 min at room temperature, under mild shaking. Optical density (O.D.) was measured at 570 nm, using 0.1*N* sodium hydroxide as blank. [26]

Statistical Analysis

Unpaired Student's *t*-tests were done for statistical analysis. Probability values of $P < 0.05$ were considered statistically

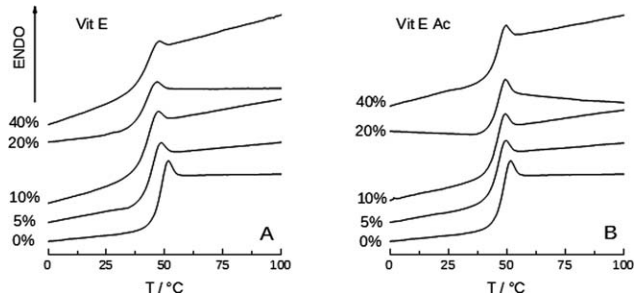

Figure 2. DSC second heating at 10°C/min for various samples with (A) Vit. E and (B) Vit. E Ac.

significant. Data were expressed as mean values ± standard deviation (S.D.).

RESULTS AND DISCUSSION

The aim of this study was to investigate the effects of Vit. E and Vit. E Ac addition to P(D,L)LA films in terms of mechanical properties and biocompatibility. The use of Vit. E, and its acetylated form, was suggested by the anti-oxidative and antinflammatory properties of these agents, which can potentially be used to prevent diseases associated with oxidative stress. Recent studies have demonstrated that oxidative stress can stimulate osteoblasts apoptosis and can reduce osteoblastogenesis. [27] Excessive accumulation of reactive oxygen species leads to cellular damage via peroxidation of lipid membrane, proteins and nucleic acids; moreover, they are also involved in the formation and activation of osteoclasts and consequently bone resorption. These findings suggest a positive effect of Vit. E on bone tissue by preventing bone loss. [19,27]

In our laboratory, P(D,L)LA films enriched with Vit. E and Vit. E Ac (5–40% w/w) have been produced.

The thermal properties of the various P(D,L)LA films and relative composites were investigated with DSC. Figure 2 reports the second heating at 10°C/min for various samples. P(D,L)LA is an amorphous polymer having a random distribution of both isomeric forms of lactic acid and accordingly is unable to arrange into a crystalline organized structure. For polylactic acid polymers, the glass transition ranges from 30°C to 60°C and increases with molecular weight and depends on the ratio

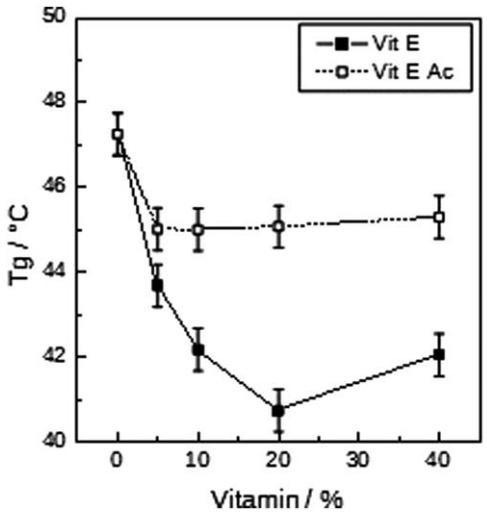

Figure 3. Trend of glass transition at second heating (10°C/min): Vit. E (fully symbols) and Vit. E Ac (open symbols).

of stereoisomers of lactide.[28] The glass transition of P(D,L)LA pure sample occurs at 47°C, as reported in the literature.[29] Using Vit. E, the glass transition decreases with increasing amount of additive. The T_g of the sample with 40% of Vit. E was 42°C (Figure 3 and Table I). Using Vit. E Ac, the glass transition is independent by the amount of additive and decreases to 45°C. The T_g depression is also obtained using biocompatible plasticizers as citrate ester,[30] glycerol,[31] or blending with other polymers,[32,33] suggesting the plasticizer effect of both Vit. E and Vit. E Ac.

In order to investigate the effect of the Vit. E and Vit. E Ac addition on the modulus and its ultimate mechanical properties, native, Vit. E and Vit. E Ac enriched P(D,L)LA films were subjected to a stress–strain analysis at a low shear rate. Figure 4 reports the stress–strain curves for the composites with Vit. E and Vit. E Ac, respectively. The tensile testing results for P(D,L)LA film and relative composites are shown in Figure 5 and Table I. Stress, strain at break, and Young's modulus of P(D,L)LA film prepared by solvent casting were 40.0 MPa, 60.0%, and 450 MPa, respectively, according to the literature.[34] Young's modulus as well as the tensile strength of both sample series increase with the increase of vitamin content until 10%

Table I. Thermomechanical Behavior for Various Samples

Samples	T_g (°C)	Young's modulus (MPa)	Yield strength (MPa)	Yield strain (%)	Stress at break (MPa)	Strain at break (%)
P(D,L)LA	47.3 ± 0.5	453 ± 21	34.4 ± 1.8	9.1 ± 0.8	38.4 ± 1.4	59.8 ± 2.1
P(D,L)LA + Vit. E 5%	43.7 ± 0.5	1070 ± 60	91.6 ±2.9	9.2 ± 0.7	83.2 ± 2.1	44.6 ± 1.6
P(D,L)LA + Vit. E 10%	42.2 ± 0.5	805 ± 38	90.1 ± 3.2	18.3 ± 1.3	84.1 ± 2.3	32.4 ± 1.3
P(D,L)LA + Vit. E 20%	40.8 ± 0.5	440 ± 25	51.4 ± 2.7	14.5 ± 1.2	33.5 ± 1.1	60.2 ± 2.5
P(D,L)LA + Vit. E 40%	42.1 ± 0.5	649 ± 32	43.5 ± 1.9	12.2 ± 1.0	43.9 ± 1.5	83.8 ± 2.9
P(D,L)LA + Vit. E Ac 5%	45.0 ± 0.5	659 ± 29	51.1 ± 2.4	9.1 ± 0.6	43.5 ± 1.3	22.6 ± 1.4
P(D,L)LA + Vit. E Ac 10%	45.0 ± 0.5	837 ± 36	73.1 ± 2.8	11.4 ± 0.9	62.6 ± 2.4	50.3 ± 2.3
P(D,L)LA + Vit. E Ac 20%	45.1 ± 0.5	465 ± 22	36.6 ± 1.7	9.5 ± 1.1	35.7 ± 1.5	93.3 ± 4.3
P(D,L)LA + Vit. E Ac 40%	45.3 ± 0.5	351 ± 15	43.5 ± 1.9	15.6 ± 1.8	45.6 ± 1.7	75.0 ± 3.4

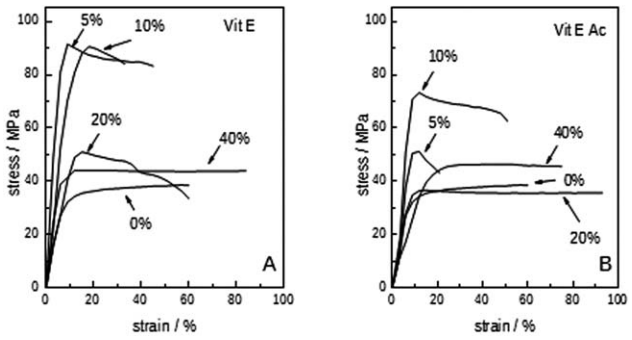

Figure 4. Stress–strain curve at 0.01s-1 for P(D,L)LA composites with (A) Vit. E and (B) Vit. E Ac.

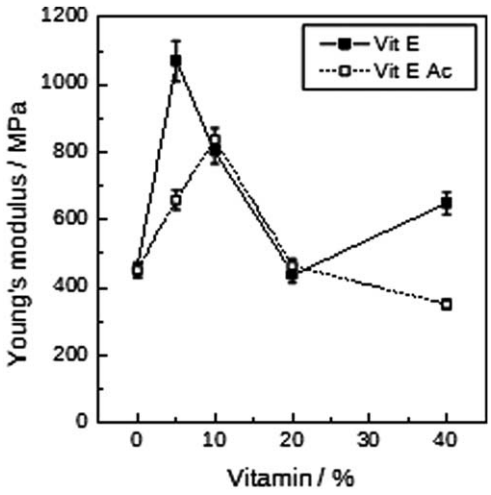

Figure 5. Trend of Young's modulus as a function of Vitamin amount: Vit. E (fully symbols) and Vit. E Ac (open symbols).

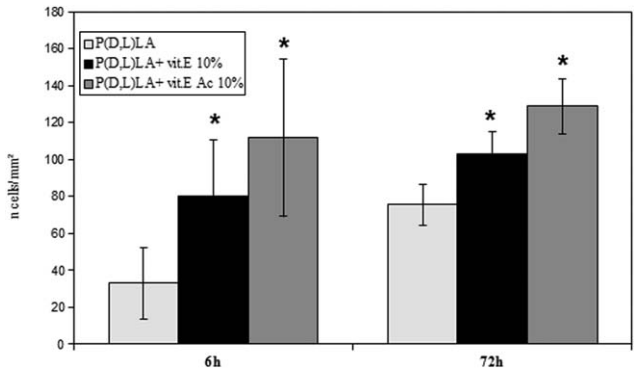

Figure 6. Evaluation of HFF adhesion onto control and Vit. E and Vit. E Ac enriched polymers. Adherent cells were counted in 10 different fields per sample at 10× magnification. $^{*}P < 0.05$

and a parallel decrease in strain at break is registered. When the amount of additive exceeded 10%, Young's modulus and stress at break of composites are comparable to pure matrix and the elongation at break increases. As observed for the thermal properties, P(D,L)LA blending with Vit. E and Vit. E Ac resulted in a significant change of mechanical properties as reported in literature for systems like PLA/PEG or PLA/PEPG[32] and PVA/Hyaluronic acid[35].

In order to make P(D,L)LA more suitable for orthopedic applications, its toughness should be improved. Vit. E and Vit. E Ac addition in a specific range (5–10%) improves P(D,L)LA mechanical properties for this kind of application, as a bone substitutes should be strong enough to fulfill the required load-bearing functions, thus high tensile strength as well as high Young's modulus are required to match the mechanical properties of the native tissue. [36,37]

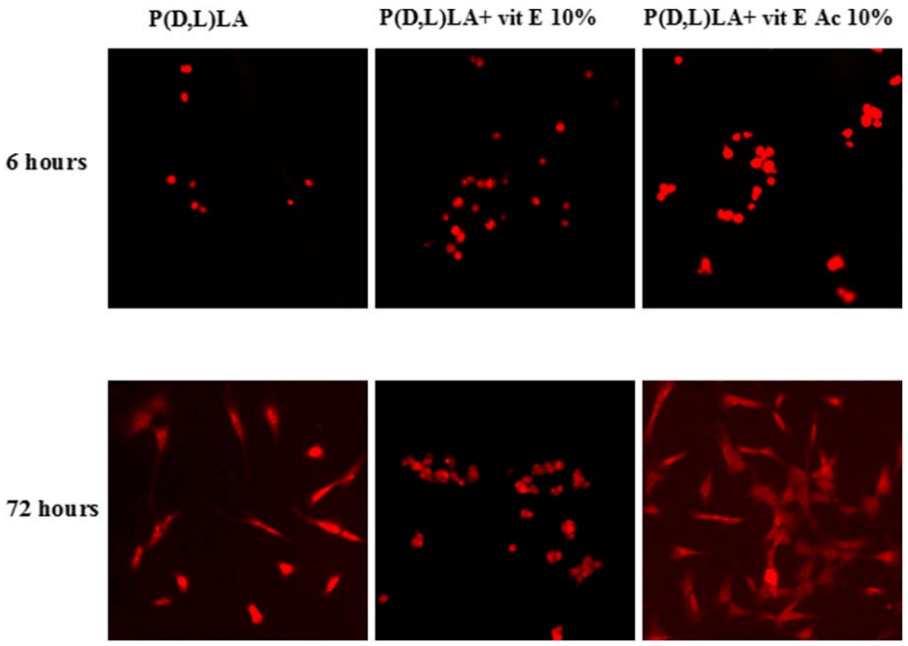

Figure 7. Representative fluorescent images of Acridine Orange stained HFF cells seeded onto control P(D,L)LA and P(D,L)LA enriched with 10% Vit. E and Vit. E Ac after 6 and 72 h. Magnification = 10×. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

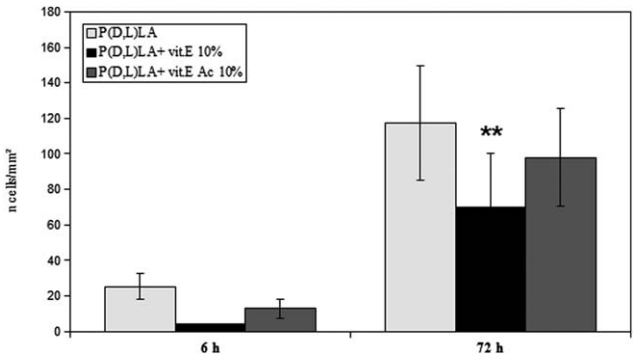

Figure 8. Cellular count of adherent MC3T3 scored onto control P(D,L)LA and P(D,L)LA enriched with 10% Vit. E and Vit. E Ac. Adherent cells were counted in 10 different fields per sample at 10× magnification. $^{**}P < 0.001$.

Implanted biomaterials surface characteristics determine host organism biologic response. In fact, both natural and synthetic materials induce both an acute and chronic local inflammatory responses, whose intensity depends on their composition, as well as on the implant site. Cell proliferation and differentiation onto implanted biomaterials surface are the major hallmark of material biocompatibility, which is the most fundamental and crucial concern for designing implantable biomaterials. [3,38–41]

As 10% Vit. E and Vit. E Ac addition seems to increase Young's modulus value in a similar way, biocompatibility assays have been performed on these samples, focusing on cell adhesion and proliferation of two different cell models, HFF and murine pre-osteoblast cells (MC3T3 E1).

As shown in Figure 6, HFF adhesion was higher onto Vit. E and Vit. E Ac enriched P(D,L)LA films. The total number of adhered HFF cells/mm^2 ± S.D. onto control P(D,L)LA films was 32.98 ± 19.11, while 80.45±30.17 and 111.7±42.6 cells/mm^2 were scored onto Vit. E and Vit. E Ac P(D,L)LA, respectively ($P < 0.05$). HFF cell proliferation was evaluated after 72 hours of incubation. The number of adhering HFF cells was significantly higher onto 10% Vit. E and Vit. E Ac enriched P(D,L)LA compared to control P(D,L)LA films. It was noteworthy that cells seeded onto control and Vit. E Ac P(D,L)LA films display an elongated morphology, whereas cells seeded onto Vit. E enriched P(D,L)LA films showed a round shape (Figure 7).

As shown in Figures 8 and 9, the difference in MC3T3 adhesion onto different polymers was statistically significant. In particular, a higher MC3T3 adhesion onto native polymer was observed as compared to both Vit. E and Vit. E Ac blended ones.

The total number of adhering MC3T3 cells/mm^2 ± S.D. onto control P(D,L)LA films was 27.02±7.62 cells/mm^2, whereas 4.25±0.1 and 13.83±5.59 cells/mm^2 were scored onto Vit. E and Vit. E Ac P(D,L)LA, respectively ($P < 0.005$).

MC3T3 proliferation was evaluated after 72 hours of incubation. The number of MC3T3 adhering onto the scaffolds was significantly decreased onto 10% Vit. E P(D,L)LA at each time point compared to that observed onto control P(D,L)LA films. On the contrary, the number of cell adhering onto 10% Vit. E Ac P(D,L)LA was similar to that observed onto control sample.

No toxic effect on HFF and MC3T3 cells (floating dead cells) was observed during daily optical inspection of cell cultures.

In previous studies, it has been demonstrated that Vit. E addition to P(D,L)LA films increased serum protein adsorption as well as surface wettability, finally resulting in a more hydrophilic polymer, probably because of the increased amount of the -OH groups introduced by the presence of Vit. E. [21] On the contrary,

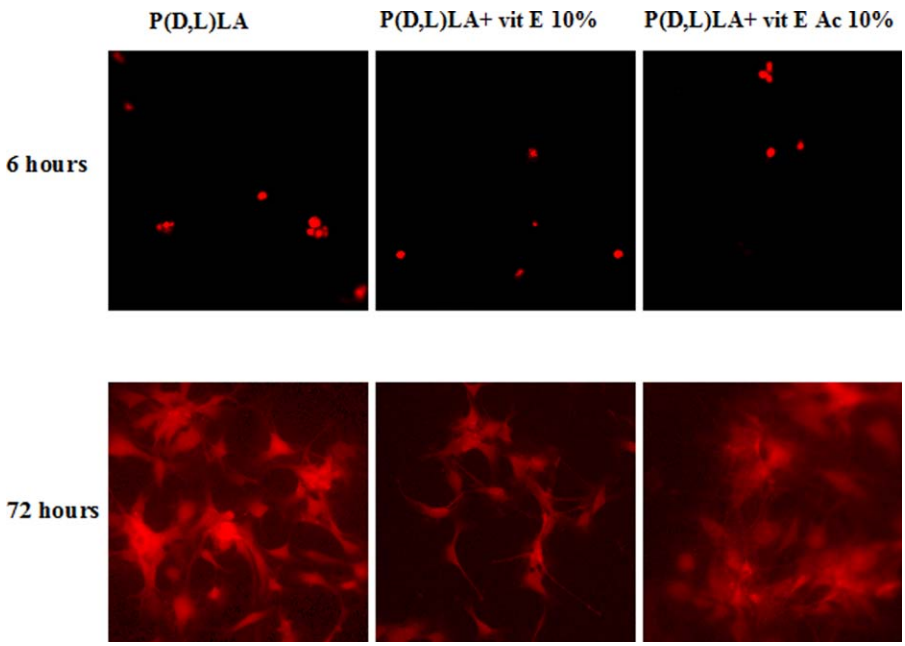

Figure 9. Representative fluorescent images of adherent MC3T3 cells seeded onto control P(D,L)LA and P(D,L)LA enriched with 10% Vit. E and Vit. E Ac after 6 and 72 h. Magnification = 10×. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

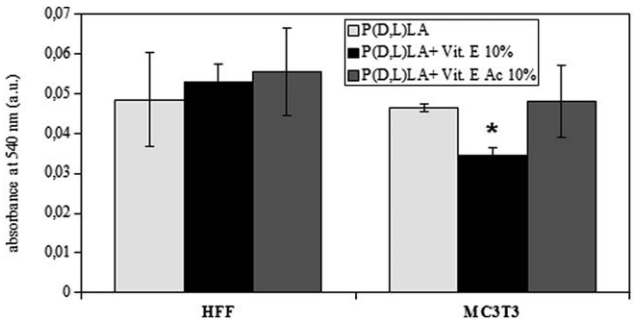

Figure 10. Sirius red quantification of collagen after 72 hours incubation onto control P(D,L)LA and P(D,L)LA enriched with 10% Vit. E and Vit. E Ac. $^*P < 0.05$

Vit. E Ac, in which the -OH groups are replaced by acetate groups, did not modify P(D,L)LA hydrophobicity. [42] Moreover, several studies demonstrated that Vit. E inhibited cell proliferation, irrespective of its antioxidant properties. [43–47]

As osteoblasts proliferation and differentiation enable the production of extracellular matrix (ECM), and as fibroblasts are the main source of connective tissue and play pivotal role in regulating ECM homeostasis, we investigate the production of collagen-containing ECM. [48]

Picro-sirius red staining was used to evaluate the secretion of the collagen-containing ECM by fibroblasts and pre-osteoblasts grown onto control, Vit. E and Vit. E Ac enriched P(D,L)LA films. As shown in Figure 10, spectrophotometric analysis confirmed the secretion of collagen by HFF and MC3T3 cells on the different polymers. Considering HFF cells, no differences were observed in collagen production when grown onto the various polymers. On the other hand, there was no difference in collagen secretion by MC3T3 cells grown onto control and 10% Vit. E Ac blended P(D,L)LA films, while there was a significant decrease in collagen secretion when cells were grown onto 10% Vit. E blended films.

CONCLUSIONS

These data demonstrate that 10% Vit. E Ac addition to P(D,L)LA films modify its mechanical behavior, improving Young's modulus as well as tensile strength, thus increasing their toughness. Moreover, this modification improves or at least did not alter P(D,L)LA biocompatibility, and could make this biomaterial more attractive for orthopedic engineering applications.

REFERENCES

1. Chandra, R.; Rustgi, R. *Prog. Polym. Sci.* **1998**, *23*, 1273.
2. Sinha Ray, S. *Acc Chem Res.* **2012**, *45*, 1710.
3. Middleton, J. C.; Tipton, A. J. *Biomaterials* **2000**, *21*, 2335.
4. Cheng, Y.; Deng, S.; Chen, P.; Ruan, R. *Fon. Chem. China* **2009**, *4*, 259.
5. Tokiwa, Y.; Calabia, B. P. *Microbiol. Biotechnol.* **2006**, *72*, 244.
6. Zhang, Z.; Feng, S. S. *Biomaterials* **2006**, *27*, 262.
7. Jacobs, T.; Declercq, H.; De Geyter, N.; Cornelissen, R.; Dubruel, P.; Leys, C.; Beaurain, A.; Payen, E.; Morent, R. *J. Mater. Sci. Mater. Med.* **2013**, *24*, 469.
8. Chen, C. C.; Chueh, J. Y.; Tseng, H.; Huang, H. M.; Lee, S. Y. *Biomaterials* **2003**, *24*, 1167.
9. Buchatip, S.; Petchsuk, A.; Kongsuwan, K. *J. Met. Mater. Miner.* **2008**, *18*, 175.
10. Chen, Q. Z.; Blaker, J. J.; Boccaccini, A. R. *J. Biomed. Mater. Res. B: Appl. Biomater.* **2006**, *76*, 354.
11. Rezwana, K.; Chena, Q. Z.; Blakera, J. J.; Boccaccini, A. R. *Biomaterials* **2006**, *27*, 3413.
12. Felfel, R. M.; Ahmed, I.; Parsons, A. J.; Rudd, C. D. *J. Mech. Behav. Biomed. Mater.* **2013**, *17*, 76.
13. Brigelius-Flohé, R.; Kelly, F. J.; Salonen, J. T.; Neuzil, J.; Zingg, J. M.; Azzi, A. *Am. J. Clin. Nutr.* **2002**, *76*, 703.
14. Pazdro, R.; Burgess, J. R. *Mech. Ageing Dev.* **2010**, *131*, 276.
15. Ricciarelli, R.; Maroni, P.; Ozer, N.; Zingg, J. M.; Azzi, A. *Free Radic. Biol. Med.* **1999**, *27*, 729.
16. Azzi, A.; Breyer, I.; Feher, M.; Pastori, M.; Ricciarelli, R.; Spycher, S.; Staffieri, M.; Stocker, A.; Zimmer, S.; Zingg, J. M. *J. Nutr.* **2000**, *130*, 1649.
17. Arjmandi, B.; Juma, S.; Beharka, A.; Bapna, M.; Akhter, M.; Meydani, S. *J. Nutr. Biochem.* **2002**, *13*, 543.
18. Turan, B.; Can, B.; Delilbasi, E. *Clin. Rheumatol.* **2003**, *22*, 432.
19. Muhammad, N.; Luke, D. A.; Shuid, A. N.; Mohamed, N.; Soelaiman, I. N. *Evid. Based Complement. Alternat. Med.* **2012**, DOI: 10.1155/2012/161527.
20. Panin, G.; Strumia, R.; Ursini, F. *Ann. N. Y. Acad. Sci.* **2004**, *1031*, 443.
21. Renò, F.; Aina, V.; Gatti, S.; Cannas, M. *Biomaterials* **2005**, *26*, 5594.
22. Renò, F.; Traina, V.; Cannas, M. *Biomol. Eng.* **2007**, *24*, 307.
23. Renò, F.; Carniato, F.; Rizzi, M.; Marchese, L.; Laus, M.; Antonioli, D. *J. Appl. Polym. Sci.* **2013**, *129*, 699
24. Cascone, M. G.; Laus, M.; Ricci, D.; Del Guerra, R. S. *J. Mater. Sci. Mater. Med.* **1995**, *6*, 71.
25. Chandrasekaran, A. R.; Venugopal, J.; Sundarrajan, S.; Ramakrishna, S. *Biomed. Mater.* **2011**. DOI: 10.1088/1748-6041/6/1/015001.
26. Tullberg-Reinert, H.; Jundt, G. *Histochem. Cell Biol.* **1999**, *112*, 271.
27. Almeida, M.; Han, L.; Ambrogini, E.; Bartell, S. M.; Manolagas, S. C. *Mol. Endocrinol.* **2010**, *24*, 2030.
28. Jamshidian, M.; Tehrany, E. A.; Imran, M.; Jacquot, M.; Desobry, S. *Compr. Rev. Food Sci. Food Saf.* **2010**, *9*, 552.
29. Li, S.; McCarthy, S. *Biomaterials* **1999**, *20*, 35.
30. Labrecque, L. V.; Kumar, R. A.; Davé, V.; Gross, R. A.; McCarthy, S. P. *J. Appl. Polym. Sci.* **1997**, *66*, 1507.
31. Martin, O.; Avérous, L. *Polymer* **2001**, *42*, 6209.
32. Jia, Z.; Tan, J.; Han, C.; Yang, Y.; Dong, L. *J. Appl. Polym. Sci.* **2009**, *114*, 1105.

33. Piorkowska, E.; Kulinski, Z.; Galeski, A.; Masirek, R. *Polymer* **2006**, *47*, 7178.

34. Tsuji, H. *Macromol. Biosci.* **2005**, *5*, 569.

35. Lazzeri, L.; Barbani, N.; Cascone, M. G.; Lupinacci, D.; Giusti, P.; Laus, M. *J. Mater. Sci. Mater. Med.* **1994**, *5*, 862

36. Hutmacher, D.; Hürzeler, M. B.; Schliephake, H. *Int. J. Oral Maxillofac. Implants.* **1996**, *11*, 667.

37. Seyedraoufi, Z. S.; Mirdamadi, S. *J. Mech. Behav. Biomed. Mater.* **2013**, *21*, 1.

38. Alves, C. M.; Yang, Y.; Marton, D.; Carnes, D. L.; Ong, J. L.; Sylvia, V. L.; Dean, D. D.; Reis, R. L.; Agrawal, C. M. *J. Biomed. Mater. Res. B: Appl. Biomater.* **2008**, *87*, 59.

39. Wang, S.; Cui, W.; Bei, J. *Anal. Bioanal. Chem.* **2005**, *381*, 547.

40. Ashammakhi, N.; Veiranto, M.; Suokas, E.; Tiainen, J.; Niemelä, S. M.; Törmälä, P. *J. Mater. Sci. Mater. Med.* **2006**, *17*, 1275.

41. Pan, H.; Jiang, H.; Kantharia, S.; Chen, W. *Biomed. Mater.* **2011**, *6*, 065002.

42. Renò, F.; Paul, G.; Rizzi, M.; Gatti, G.; Marchese, L. *J. Appl. Polym. Sci.* **2013**, *129*, 1527.

43. Agarwal, M. K.; Iqbal, M.; Athar, M. *Redox Rep.* **2005**, *10*, 62.

44. Yano, T.; Yajima, S.; Hagiwara, K.; Kumadaki, I.; Yano, Y.; Otani, S.; Uchida, M.; Ichikawa, T. *Carcinogenesis* **2000**, *21*, 2129.

45. Kempná, P. Reiter, E.; Arock, M.; Azzi, A.; Zingg, J. M. *J. Biol. Chem.* **2004**, *279*, 50700.

46. Sakamoto, T.; Hinton, D. R.; Kimura, H.; Spee, C.; Gopalakrishna, R.; Ryan, S. J. *Graefes. Arch. Clin. Exp. Ophthalmol.* **1996**, *234*, 186.

47. Azzi, A.; Boscoboinik, D.; Clément, S.; Marilley, D.; Ozer, N. K.; Ricciarelli, R.; Tasinato, A. *Prostaglandins Leukot. Essent. Fatty Acids* **1997**, *57*, 507.

48. Urban, K.; Höhling, H. J.; Lüttenberg, B.; Szuwart, T.; Plate, U. *Head Face Med.* **2012**, *8*: 25, doi:10.1186/1746-160X–8-25.