Poly(diol citrate) nanocomposites with enhanced mechanical properties

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Introduction

The mechanical properties of a tissue engineering scaffold are particularly important when engineering soft tissues as it has been shown that mechanical stimulation during *in vitro* tissue development can modulate cell differentiation, increase extracellular matrix synthesis, and enhance the mechanical properties of cartilaginous, ligamentous, and smooth muscle containing tissues [1-5]. Unfortunately, one of the main limitations with poly(hydroxyortho esters) and other polymers commonly used in tissue engineering is their inability to undergo large reversible elastic deformations when stressed [6].

Due to the stiffness and lack of elasticity of commonly used polymers in tissue engineering, our laboratory has developed a novel family of biodegradable elastomeric polyesters referred to as poly(diol citrates) [7-9]. Their mechanical properties can be adjusted depending on the selection of diols and the post-polymerization conditions [8]. Nevertheless, the mechanical properties that can be obtained may not meet the demanding requirements of musculoskeletal tissues, which are often exposed to relatively large tensile or compressive loading forces. In this study we describe the fabrication and mechanical characterization of novel elastomeric nanocomposite materials in which the macrophase consists of a poly(1,10-decanediol-co-citrate) (PDC) elastomer and the nanophase consists of a poly(L-lactic acid) (PLLA) nanofibrous network or poly(lactic-co-glycolic acid) (PLGA) nanoparticles.

Materials and Methods

Synthesis of poly(diol citrate) pre-polymer

Citric acid and 1,10-decanediol were melted under a flow of nitrogen gas by stirring at 165˚C in a silicon oil bath and then stirred for another hour at 140˚C to create a pre-polymer solution of poly(1,10-decanediol-co-citrate) (PDC). For nanocomposite preparation, the pre-polymer was dissolved in ethanol.

Fabrication of non-porous nanocomposites

The nanofibrous nanocomposite was fabricated in a two step process. Firstly, a PLLA nanofibrous network was fabricated via thermally induced gelation in tetrahydrofuran (THF) followed by solvent exchange and freeze drying [10]. Secondly, the PLLA nanofibrous network was impregnated with the PDC pre-polymer solution. After the final coating, the composite was polymerized at 80°C for 3 days without vacuum. Porous nanofibrous nanocomposite scaffolds were prepared using solvent casting and salt leaching techniques.

Nanoparticle reinforced composites were fabricated by adding PLGA nanoparticles to the PDC prepolymer prior to removal of solvent and subsequent polymerization. PLGA nanoparticles were fabricated using a modified spontaneous emulsion and solvent diffusion method [11].

Characterization of nanocomposites

Non-porous samples were tested for ultimate tensile strength, Young's modulus, and strain at break. Tensile tests were performed according to ASTM D412a on an Instron 5544 mechanical tester (Instron, Canton, MA) equipped with a 500N load cell. The compressive modulus of the salt-leached, porous scaffolds was evaluated in unconfined compression using a stepwise stress/relaxation test [12, 13].

The density and porosity of scaffolds was measured using a method based on Archimedes' principle [14]. The morphology of nanofibrous networks and nanocomposites was studied using an LEO 1525 scanning electron microscope (Zeiss, Thornwood, NY). SEM images were analyzed using image analysis software (Image-Pro® Plus V.5.0, Silver Spring, MD) to obtain the pore size and fiber diameter data.

Statistical methods

Data are expressed as means ± standard deviation. The statistical significance between two sets of data was calculated using two-tail Student's *t*-test. Analysis of variance (ANOVA) with the Tukey post-hoc test was used to determine significant differences among three or more means. Data were taken to be significant, when a P-value of 0.05 or less was obtained.

Results and Discussion

Nanofibrous networks were created with PLLA concentrations of 5% or 10%. **Table (1)** shows the characteristics of the nanofibrous networks. Increasing the PLLA concentration led to an increase in the network's density and fiber diameter while decreasing its porosity and pore size. A lower porosity decreases the amount of poly(diol citrate) macro-phase that would be incorporated into the final composite.

Table (1) – PLLA Nanofibrous Network Characteristics

	5% PLLA	10% PLLA
Fiber Diameter (nm)	246.3 ± 69.1	206.7 ± 70.4
Density (g cm^{-3})	0.063 ± 0.004	0.117 ± 0.006
Porosity (%)	94.95 ± 0.32	90.56 ± 0.50
Pore Size (nm)	643.8 ± 233.5	617.2 ± 362.9

The addition of PLLA nanofibrous networks to poly(diol citrate) elastomers resulted in a composite with increased mechanical properties. The mechanical properties and representative stress-strain curves of the composites with different percentages of PLLA are shown in **Figure (1).** The presence of a PLLA nanofibrous network provided reinforcement to the poly(diol citrate) as demonstrated by the increased tensile strength, modulus, and elongation at break. Increases in tensile strength of 150% over the PDC control and 400% that of PLLA were seen with a 10% nanocomposite. Similarly, the modulus increased by 1000% compared to PDC without PLLA reinforcement. Although the modulus of the composite decreased relative to the PLLA network, the addition of the non-elastic PLLA nano-phase increased the elongation at break from 200% for the PDC control to 300% for the 10% nanocomposite. In contrast, the elongation at break for PLLA controls was less than 5%. PDC nanocomposites could be elongated to a much greater length with little permanent plastic deformation. The amount of permanent deformation after break of PDC scaffolds was less than 4%. For the tensile strength, modulus, and elongation, a statistically significant difference was found when comparing the PDC control (no PLLA) and the PLLA-PDC nanocomposites, confirming that the mechanical properties could be significantly increased using nanofibrous composite elastomers. Comparing between composites reinforced with different amounts of

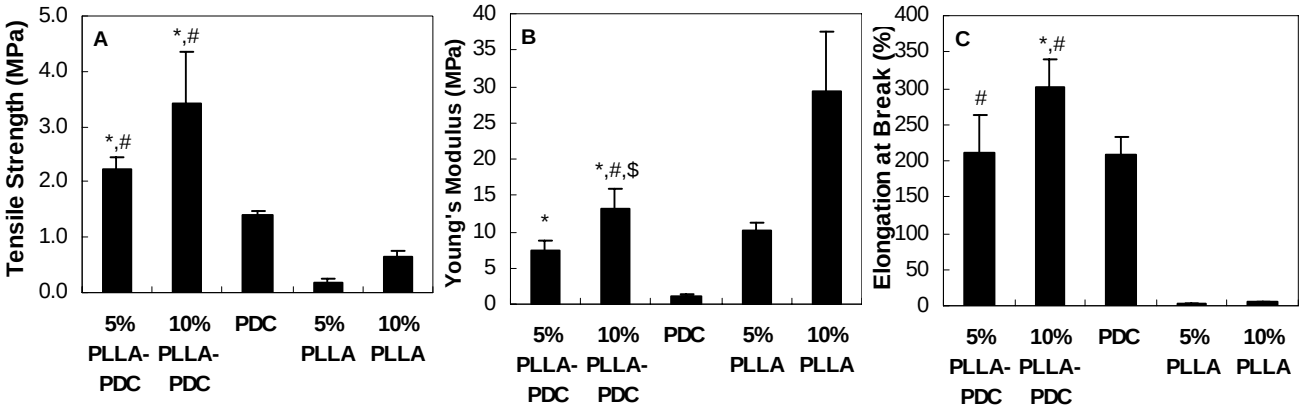

Figure (1) – (A) Tensile strength, **(B)** Young's modulus, **(C)** Elongation at break of PLLA-PDC nanocomposites and controls that were polymerized at 80°C for 3 day without vacuum (n = 4). (* $p<0.05$ from PDC control, # $p<0.05$ from PLLA control, $ $p<0.05$ comparing 5% PLLA-PDC to 10% PLLA-PDC)

PLLA, there is an increase in the mechanical properties with increasing PLLA concentration from 5% to 10% (**Figure 1**). The tensile strength, Young's modulus, and elongation at break increased by 54%, 75%, and 44%, respectively. The increase in mechanical properties with the addition of PLLA nanofibrous networks may be due to mechanical interlocking and/or interactions between the nanofibers and the elastomer chains.

Since poly(diol citrates) composites are targeted for tissue engineering, porous nanocomposites scaffolds were created through the incorporation of salt particles during the gelation step. Both the 5% and 10% nanocomposites exhibited an open and interconnected pore structure with high porosity. This feature is beneficial for tissue engineering applications as a three dimensional highly porous structure is required to support cell attachment, proliferation, and extracellular matrix synthesis. More importantly, the scaffold should possess mechanical properties that match the host tissue at the site of implantation. The compressive modulus increased with increasing PLLA concentration in the nanocomposite (**Figure 2**). For the 10% PLLA- PDC concentration, the compressive modulus was 439 ± 106 kPa, similar to that of human (581 ± 168 kPa) and bovine (310 ± 180 kPa) articular cartilage [12, 13]. In addition, a statistically significant difference was found when comparing the PDC control scaffolds to the PLLA-PDC nanofibrous scaffolds.

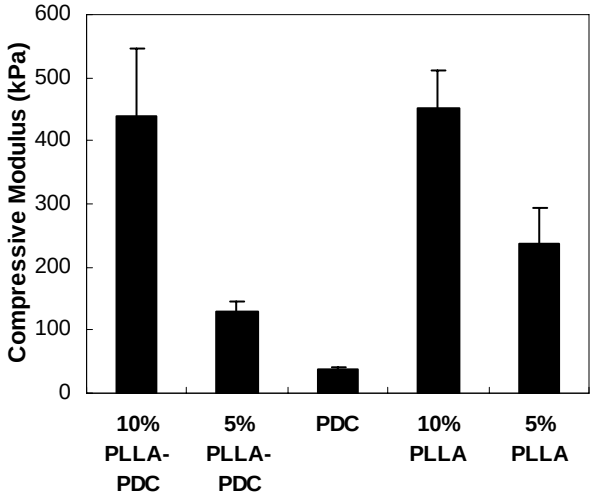

Figure (2) – Compressive modulus of PDC-PLLA nanofibrous scaffold composites. (* $p<0.05$ from PDC control, # $p<0.05$ from PLLA control, $ $p<0.05$ comparing between 5% PLLA-PDC and 10% PLLA-PDC)

Nanocomposites were also fabricated using PLGA nanoparticles (Diameter = 178 nm, Polydispersity = 0.005). Nanocomposites fabricated with PLGA nanoparticles showed an

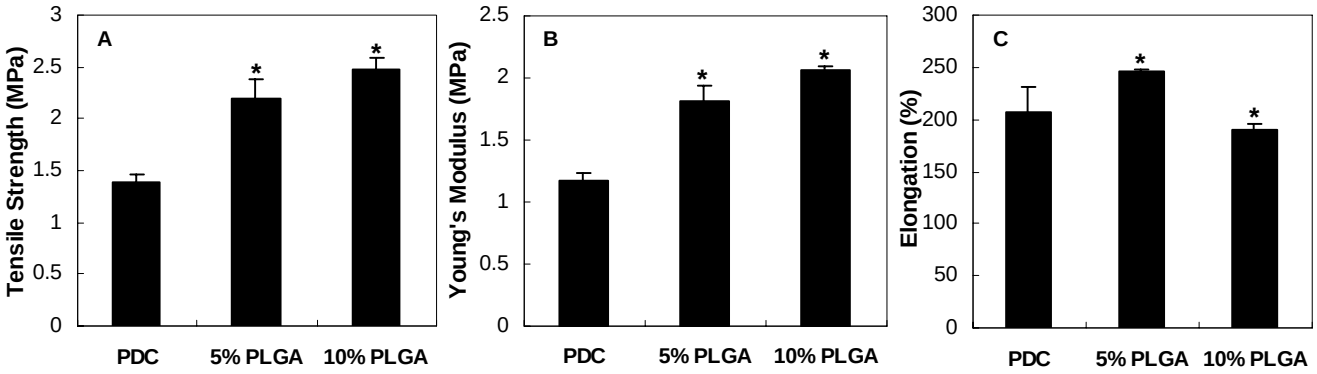

Figure (3) – (A) Tensile strength, **(B)** Young's modulus, **(C)** Elongation at break of PLGA-PDC nanocomposites that were polymerized at 80°C for 3 day without vacuum (n = 4). (* $p<0.05$ from PDC control)

increase in mechanical properties with increasing nanoparticle concentration (**Figure 3**). For a 10% PLGA-PDC nanoparticle composite, the modulus and tensile strength increased by 116% and 40% respectively over the control while the elongation at break decreased from 372% to 190%.

Conclusion

Novel nanocomposite elastomers, using a poly(1,10-decanediol-co-citrate) elastomeric macrophase and a nanophase consisting of a nanofibrous network of poly(L-lactic acid) or poly(lactic-co-glycolic acid) (PLGA) nanoparticles, were fabricated for potential use in soft tissue engineering. The incorporation of a nanophase into the PDC elastomer increased the mechanical properties while still maintaining elasticity. In addition, it was demonstrated that the mechanical properties could be adjusted by varying the concentration of the nanophase. The range of mechanical properties of tissue engineering scaffolds approached that of human and bovine cartilage. To our knowledge, this is the first report of a nanocomposite material for tissue engineering where the macro and nano phases were made from biodegradable and biocompatible synthetic co-polymers.

References

1. Kim, B.S. and D.J. Mooney, *Scaffolds for engineering smooth muscle under cyclic mechanical strain conditions.* J Biomech Eng, 2000. **122**(3): p. 210-5.
2. Nerem, R.M., *Role of mechanics in vascular tissue engineering.* Biorheology, 2003. **40**: p. 281-287.
3. Niklason, L.E., et al., *Functional arteries grown in vitro.* Science, 1999. **284**(5413): p. 489-93.
4. Waldman, S.D., et al., *Effect of biomechanical conditioning on cartilaginous tissue formation in vitro.* J Bone Joint Surg Am, 2003. **85A**(Suppl 2): p. 101-105.
5. Kim, B.S., et al., *Cyclic mechanical strain regulates the development of engineered smooth muscle tissue.* Nature Biotechnology, 1999. **17**(10): p. 979-83.

6. Yang, S., et al., *The design of scaffolds for use in tissue engineering. Part I. Traditional factors.* Tissue Engineering, 2001. **7**(6): p. 679-689.
7. Yang, J., A. Webb, and G. Ameer, *Novel citric acid-based biodegradable elastomers for tissue engineering.* Advanced Materials, 2004. **16**(6): p. 511-516.
8. Yang, J., et al., *Synthesis and evaluation of novel biodegradable elastomeric polyesters.* Biomaterials, 2006. **27**: p. 1889-1898.
9. Yang, J., et al., *Modulating expanded polytetrafluoroethylene vascular graft host response via citric acid-based biodegradable elastomers.* Advanced Materials, 2006. **18**(12): p. 1493-1498.
10. Ma, P. and R. Zhang, *Synthetic nano-scale fibrous extracellular matrix.* J. Biomed Mater Res, 1999. **46**: p. 60-72.
11. Murakami, H., et al., *Preparation of poly(DL-lactide-co-glycolide) nanoparticles by modified spontaneous emulsifcation solvent diffusion method.* International Journal of Pharmaceutics, 1999. **187**: p. 143-152.
12. Jurvelin, J.S., M.D. Buschmann, and E.B. Hunziker, *Mechanical anisotropy of the human knee articular cartilage in compression.* Proc Inst Mech Eng [H], 2003. **217**(3): p. 215-9.
13. Korhonen, R.K., et al., *Comparison of the equilibrium response of articular cartilage in unconfined compression, confined compression and indentation.* J Biomech, 2002. **35**(7): p. 903-9.
14. Yang, J., et al., *Fabrication and surface modification of macroporous poly(L-lactic acid) and poly(L-lactic-co-glycolic acid) (70/30) cell scaffolds for human skin fibroblast cell culture.* Journal of Biomedical Materials Research, 2002. **62**(3): p. 438-46.