

New properties from PLA–PEO–PLA hydrogels

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Polymeric materials are important in many medical applications. Regenerative medicine offers the potential to repair or replace damaged tissue and polymers are an essential component of many tissue engineering approaches. Hydrogels have many advantageous properties but, generally, lack robust mechanical properties. At the same time, mounting evidence points to the importance of the matrix modulus when constructing devices. In this context, triblock copolymers made from poly(L-lactide)–poly(ethylene glycol)–poly(L-lactide) have been prepared and formulated into hydrogels. Investigations into their mechanical properties found the elastic modulus to be greater than 10 kPa which is at least one order of magnitude stiffer than previously reported from macromolecules composed of similar monomers. Part of the reason is the presence of crystalline lactide domains. Creating hydrogels with tailored modulus across the kPa range will likely have important ramifications in regenerative medicine.

Introduction

One of the most prevalent human health problems is the loss or failure of an organ or tissue system. As a result, progress in the area of regenerative medicine is critical and will dramatically impact global healthcare. One part of this broader topic focuses on the use of polymeric materials as the critical element in tissue engineering devices. Because of the severe shortage of organs for transplantation, tissue engineering has received significant attention in meeting supply demands.^{1,2} In general, these devices serve to regenerate

tissues and are typically composed of a 3D polymeric scaffold, which attempts to initially replace the natural extracellular matrix, and cells of the desired type (a specific cell type or stem cells).³ Studies have looked at essentially every tissue with significant work performed in the area of skin, blood vessels, cartilage, liver, and kidney regeneration.⁴ Regenerative medicine using polymeric scaffolds seeded with the patient's own cells are now at or near clinical use.^{3,5,6} The polymeric scaffold would ideally mimic many of the roles assumed by the extracellular matrix including overall shape, tissue structure, cell regulation, mechanical response, diffusion of nutrients, metabolites, and growth factors.

Despite much success from early polymeric scaffolds, it becomes increasingly clear that bulk porous polymers, like polyesters, are relatively poor examples of extracellular matrix. They do not have similar mechanical properties and are chemically much simpler. Alternatively, hydrogels appear to be

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Table 1 Common scaffolds used for tissue engineering along with their compressive and elastic moduli

Material	Compressive modulus/kPa	Elastic modulus/kPa ^a
Alginate ³⁰	5	1.7
Agarose ^{31,32}	8	2.7
Chitosan ^{33,34}	3–30	1–10
Collagen ^{35,36}	0.1–1	0.03–0.3
Fibrin glue ^{37,38}	5	1.7
PEO-co-PPO ^{39,40}	0.6	0.2
KDL peptide ⁴¹	1	0.3
PGA ²⁰	1	0.3

^a Estimated for some materials from measured compressive modulus for comparison with elastic modulus of materials discussed here.

much better candidates even if many of them remain unfunctionalized from a chemical or chemical signalling point of view.^{3,7} Hydrogels at least have hydrophilicity, open pore structures, surface tension, and, in some cases, mechanical properties in common with native tissue, unlike the most popular polymers used currently. Hydrogels have been created from a wide variety of materials, some of which are listed in Table 1.

The ability of cells to respond to chemical signals is well documented. At the same time, there is mounting evidence that mechanical cues can have similarly large influences on cellular response. It is known that cells have bio-mechanical response systems and so the fact that the matrix material can significantly impact cellular cycles and behavior is logical. Studies aimed at understanding the influence of matrix mechanical properties remain scarce compared to those studying chemical signals; however, more laboratories are beginning to address this aspect. Mechanical properties impact cellular structure, metabolism, transcription and/or translation of various genes, and even viability.^{8–14} Pioneering work showed NIH3T3 fibroblasts, a commonly studied cell line, and rat kidney epithelial cells underwent compliance-dependent motility and cytoskeletal adhesion changes.^{13,15} The ability of substrate compliance to impact cell movement, or motility, is striking. Other studies have looked at endothelial cells, myocytes, hepatocytes, neural/glial cells, and chondrocytes.^{16–20}

Myoblasts formed myotubes but only exhibited striations on intermediate stiffness substrates.¹⁷ Amazingly, the modulus of healthy muscle tissue is within this intermediate range of stiffness. Glial cells, unlike neurons, were unable to survive in soft materials, an important distinction for designing materials to repair central nervous system injuries.^{16,18,21,22} Further, the use of hydrogels has been proposed to limit the level of scar tissue as a result of mechanical properties at the wound site.¹⁶

Using cartilage as an illustrative example, the diseases of cartilage in various forms, including osteoarthritis and intervertebral disc degeneration, are the leading cause of disability in the US, with annual costs exceeding \$65 billion. Over one million procedures per year involve cartilage reconstruction.² Diseases of cartilage are particularly problematic given the lack of innate repair capacity of the tissue, which is, at least partially, related to its lack of vascularity and relatively low cell density. The use of a 3D matrix is critical in the case of chondrocytes, as shown by cultures on 2D surfaces where the cells dedifferentiate, assume a flattened morphology, and produce type I instead of type II collagen.²³ A variety of scaffold materials have been explored including collagen, alginate, hyaluronic acid, and many synthetic polymers (see Table 1).^{3,23–29} The basic principles of tissue scaffolds for cartilage repair have shown exceptional promise although many aspects of the device (cell lines, polymer scaffold, *etc*) remain active areas of research with significant improvements still required. In this article, we focus solely on our recent efforts to design physically cross-linked hydrogels based on PLA-PEO-PLA block copolymers with increased stiffness compared to current materials like pluronics and other hydrogels shown in Table 1. We discuss the interrelationships between polymer composition, mechanical properties, and hydrogel structure.

Lactic acid–ethylene oxide polymers as hydrogel-based biomaterials

Polyesters remain a candidate for many biomaterials applications from small area tissue repair to the growth of new organs. The chemical structures of three polyesters are shown



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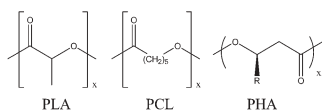


Fig. 1 Three polyesters used as tissue engineering scaffolds.

in Fig. 1. The overwhelmingly attractive feature of these materials is their biodegradation and ease of synthesis. However, in relation to the optimal requirements of a scaffold, these polymers are far from ideal. They are very hydrophobic which makes compatibility with biological environments poor. Although PLA materials are technologically important, they do suffer from unwanted responses *in vivo* including foreign body response.⁴² At the same time, the modulus of homopolymers can be quite far from those of biological tissues. Although PLA and other synthetic polyesters, like poly(ϵ -caprolactone), represent important materials for medical implants, they all suffer from a variety of critical limitations. Another class of polyesters, which has seen limited exploration in biomedical applications, is poly(hydroxyalkanoates) (PHAs).⁴³ Preliminary experiments performed *in vivo* strongly suggest PHAs are viable polymers for biomedical applications.^{43,44} Vascular grafts coated with poly(hydroxyoctanoate) (PHO) were implanted into rats and dogs and showed no adverse inflammatory reactions and moderate-to-good healing responses.⁴⁵ In addition, PHO coated implants exhibited similar healing characteristics compared to widely-used and commercially available protein impregnated grafts including gelatin, cross-linked gelatin, and cross-linked albumin.^{45,46}

One approach to enhancing the water-solubility of these polyesters is to make block copolymers containing a water soluble block such as polyethylene oxide (PEO).⁴⁷ As a result of the increased water solubility of these materials provided by PEO, they form physically cross-linked hydrogels through associates of the hydrophobic blocks.⁴⁸ We confine ourselves to copolymers based on biodegradable hydrophobic poly(lactic acid) (PLA), including the two stereoisomers L and D, and hydrophilic poly(ethylene oxide) (PEO). Beyond the control of block lengths, which is known to influence properties including modulus as discussed below, the ability to control D,L stereochemistry directly impacts hydrogel modulus through crystallinity of the PLA blocks since pure L or D polymers are semi-crystalline but mixtures of D and L produce amorphous materials.

Block copolymers based on LA and EO segments have attracted considerable attention over the last decade. Diblock copolymers based on these two segments were first reported in 1987 by Cohn and co-workers who studied the morphology and *in vitro* degradation.^{49–61} In 1997, Kim and co-workers reported the discovery of copolymers with liquid–gel transitions at body temperature and *in vivo* delivery by injection, although the polymer architecture was inverted to PEO–PLA–PEO.⁶² This report by Kim, along with renewed interest in hydrogels, has created broader interest in materials composed of LA and EO.^{63,64} Much of this early work focused on materials containing very little water. In fact, many systems have been described as hydrogels but are actually composed of as much as 80 wt% polymer.

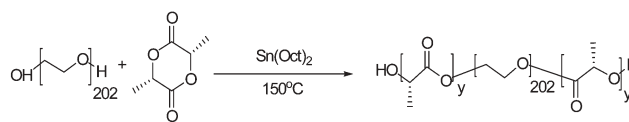


Fig. 2 Bulk polymerization of PEG with L-lactide at 150 °C.

Table 2 Molecular weight characteristics of PLLA–PEO–PLLA triblock copolymers

Sample	$M_{n\text{PEG}}^a$	$M_{n\text{PLLA}}^b$	M_{Total}	Total $\text{DP}_{\text{PLLA}}^b$	MWD ^c
1	8900	3744	12 644	52	1.24
2	8900	5184	14 084	72	1.20

^a Determined by MALDI-TOF and GPC; ^b Determined by ¹H-NMR. ^c Determined by GPC.

The copolymers described here were prepared by ring-opening polymerization of L-lactide at 150 °C in the bulk using stannous(II) 2-ethylhexanoate as catalyst as shown in Fig. 2. Although we have prepared a number of copolymers, two representative examples are shown here in which the PEO block length is held constant and the degree of polymerization (DP) for PLLA is varied from 52 to 72.^{47,48} ¹H-NMR integration was used to establish the M_n for the PLLA blocks as opposed to GPC standards. In all cases, the polymerization was not run to completion since this broadens the molecular weight distribution (MWD) (Table 2). Thermal and diffraction studies of these polymers in the bulk always show PEO crystallization as well as PLA crystallization when the block length is sufficiently long.⁶⁵ When both blocks crystallize, the PEO segments are confined between PLA lamellae.⁶⁵

Mechanical properties of these hydrogels

Addition of water to these block copolymers produces hard, physically associated gels typically above 16 wt%. Fig. 3 shows the material near the sol–gel boundary in which 14 wt% is a viscous liquid while 16 wt% creates a stiff gel. These physically associated networks are analogous to reversible network gels formed from telechelic hydrophobically modified polymers. Characteristics of gel formation were seen to vary with the length of the hydrophobic blocks. Solutions of the triblock with the smallest hydrophobes (**1**) did not gel up to concentrations of 20 wt% polymer, while those with longer PLLA blocks, like **2**, gelled at moderate concentrations (16 wt%). The gels formed were strong enough to support

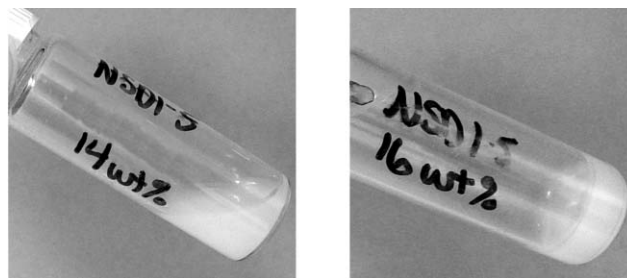


Fig. 3 Photographs at room temperature of two samples below and above the sol–gel boundary, respectively. The 14 wt% sample flows easily compared to the 16 wt% sample which is stiff.

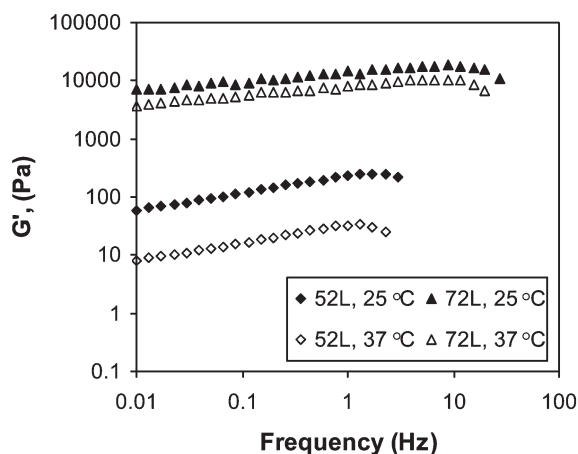


Fig. 4 Elastic modulus for hydrogels of **1** and **2** at 20 wt% and 16 wt%, respectively. The filled and open symbols were collected at 25 °C and 37 °C. The stiff 18 kPa gel is less sensitive to temperature changes than the softer materials made from **1** with shorter PLLA blocks.

their own weight, and the stir bar, when kept upside down for long periods of time. To better quantify the mechanical properties of these hydrogels, dynamic mechanical rheology was employed. Fig. 4 shows G' vs. frequency for the two polymers described above at 25 °C (filled symbols) and 37 °C (open symbols). The gel of **1** was prepared in 20 wt% but still forms a weaker gel than **2** which only contains 16 wt% polymer. It is clear from these results that the hydrophobic length, or DP, significantly influences elastic modulus. Comparing these two materials, the stiffness increases by more than two orders of magnitude, from ~ 100 Pa to 10 000 Pa, as the length of the hydrophobic block is increased.

The gels of **2** are quite elastic, with G' only weakly dependent on frequency and greater than G'' over the entire frequency range (not shown). PEO containing alkyl hydrophobe end-caps show qualitatively similar trends for the high-frequency limit of G' ,⁶⁶ although the dependence of G' on hydrophobe length is weaker for the alkyl-capped systems than in these PLLA–PEO–PLLA gels. In addition, the high-frequency elastic moduli of fluoroalkyl-capped PEO gels were insensitive to hydrophobe length.^{67,68} Although more work is necessary to fully understand the subtleties of the PLLA–PEO–PLLA system, these differences might be related to the crystalline nature of the PLLA hydrophobic domains. WAXD studies on these gels show strong diffraction peaks at $2\theta = 17^\circ$ and 19° corresponding to crystalline PLLA (Fig. 5). At the same time, the diffraction signals observed for PEO in the bulk material are absent in the hydrogel, consistent with hydration of these segments upon hydrogel formation.

The ability to create gels with elastic modulus greater than 10 kPa provides materials that are almost an order of magnitude stiffer than any previous reports for polymers composed of similar chemistry or those in Table 1. Recently, a copolymer of PMG₁₉–PEO₃₃–PMG₁₉ (PMG = poly(D,L-3-methyl-glycolide)) was reported with elastic modulus less than 500 Pa for a 27 wt% sample.⁶⁹ Kimura and co-workers reported hydrogel formation from stereocomplexed PLLA₁₈–PEO₁₀₅–PLLA₁₈ and PDLA₁₅–PEO₁₀₅–PDLA₁₅ in which 10 wt% solutions had an elastic modulus up to 1000 Pa at

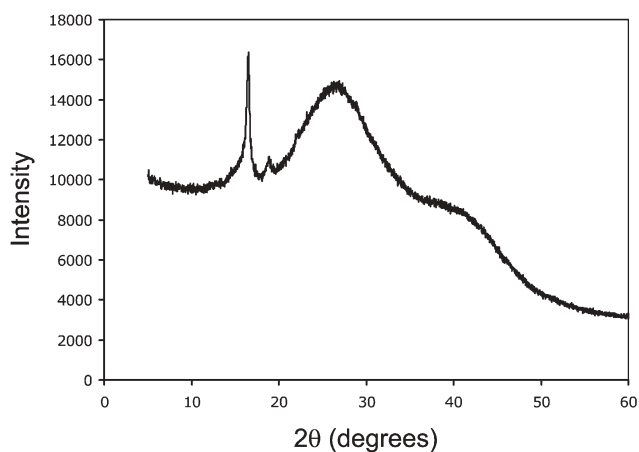


Fig. 5 WAXD of a 25 wt% gel showing strong diffraction peaks at 17° and 19° corresponding to crystalline PLLA.⁶⁵ Signals from PEO present in the bulk are absent in this highly hydrated gel. These diffraction peaks confirm the presence of crystalline PLLA in the gel.

37 °C.⁷⁰ Solutions of either polymer independently at 10 wt% did not form a hydrogel. These reports have focused on relatively small molecular weight (MW) polymers, which are quite different from those reported here. Further, the data is taken at a single frequency and strain, so the dependence of modulus on frequency and strain is completely unknown.⁴⁷

Creating hydrogels with modulus values in the kPa range are of widespread interest since many native tissues have moduli in this range, although most have nonlinear response to strain. For example, the modulus of several tissues is listed: human nasal cartilage (234 ± 27 kPa),^{71,72} bovine articular cartilage (990 ± 50 kPa),^{71,72} pig thoracic aorta (43.2 ± 15 kPa),⁷³ nucleus pulposus and eye lens ($\sim 10^3$ Pa),⁷⁴ and canine kidney cortex (~ 10 kPa).⁷⁴ These values show the need for hydrogels with stiffer modulus values than those in Table 1. At the same time, the modulus of cartilage remains at the very high end of these values, a challenging goal that needs to be met.

Gel structure

Stiff modulus, an opaque appearance, and the presence of crystalline domains for the stereo-regular PLLA–PEO–PLLA polymers suggested these materials might not form small nanometre sized spherical micelles as expected, whereas the polymers made using a racemic mixture of D and L LA blocks would form micelles owing to the presence of amorphous domains in the triblock polymer and a clearer appearance. Small angle neutron scattering and confocal microscopy experiments on a large series of stereo-regular and racemic samples confirmed this hypothesis.⁷⁵ Gels formed from amorphous, racemic PLA showed a clear signal in SANS associated with 8–10 nm spherical micelles. We do not observe large scale structures in these gels *via* confocal microscopy indicating that the polymer gel is a continuous matrix of bridged micelles with no micron size inhomogeneities present in the system. In contrast, gels formed from identical DP, stereo-regular polymers exhibited scattering consistent with poly-dispersed crosslinked structures, with length scales ranging from nano- to micrometres. A hydrophobically stained

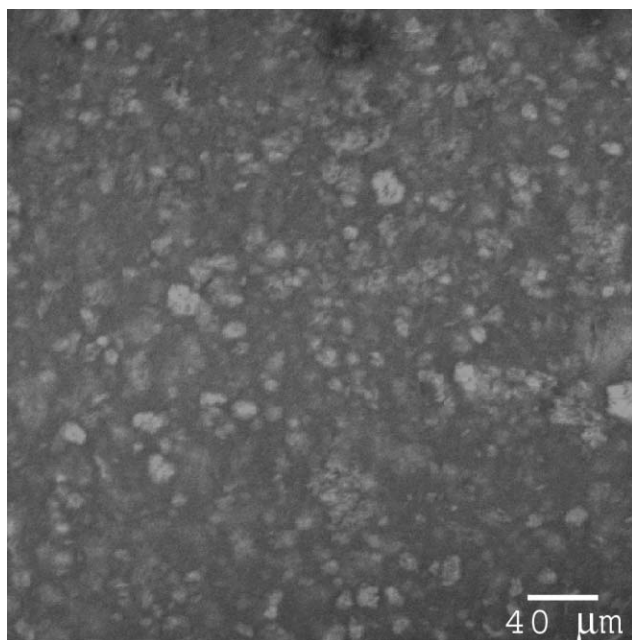


Fig. 6 Confocal microscopy image of a 25 wt% gel made from **2**.

confocal microscopy image of the gel formed by **2** is shown in Fig. 6. The brighter spots in the micrograph indicate the regions where the hydrophobic fluorescent dye preferentially segregates, which are most likely to be the regions where network junctions are located. The micrograph shows that the hydrogel has interspersed inhomogeneous regions of hydrophobic microdomains with water channels running between them. This can have important implications for drug delivery and tissue engineering applications as the presence of water channels throughout the hydrogel can easily facilitate transport of drugs or nutrients in and out of the matrix and provides space for cell encapsulation. Image analysis, using ImageJ software, was performed on this micrograph and yields a size distribution between 0.4–22 μm with the mode of the size distribution lying at approximately 0.5–2 μm .

Conclusion

Stiff hydrogels composed of PLLA–PEO–PLLA triblock copolymers have been described. Although these materials have been known for well over 20 years, new properties have been discovered by the judicious choice of MW, composition, and stereochemistry. Control over crystallinity significantly influences gel stiffness and structure, representing a new macromolecular parameter for hydrogel design. These materials have the ability to support their own weight in simple device constructs, which might allow molding applications to create intricately shaped monoliths. The production of designed monoliths with varying size and shape is a limitation of routine scaffolds currently available for medical applications. It remains unlikely that a single material will ever meet the various design parameters of all applications further highlighting the need for continual exploration of a wide range of polymeric materials to meet the growth demands of regenerative medicine. Application of the design principles

discussed here to PHA–PEO–PHA materials is currently underway to determine if they are universal as well as to combine the excellent properties of hydrogels with the encouraging biodegradation response of PHAs. Although we show that modulus can be controlled, the upper kPa range has not yet been achieved. At the same time, these materials appear to require invasive procedures since they do not exhibit phase transitions in the appropriate temperature range. Their dissolution properties remain a potential concern and the long term performance is also unknown.

Obtaining materials that exhibit nonlinear mechanical properties, a feature of many soft tissues, remains to be explored. This behavior is exhibited by a variety of biopolymer gels including gelatin,⁷⁶ keratin,⁷⁷ filamin,⁷⁸ and fibrin,⁷⁹ but is not typically seen in gels of synthetic block copolymers. This feature is thought to be due in part to the rigidity of microdomains within these gels, which we can potentially reproduce by varying the crystallinity of the PLA domains. Finally, more hydrogels, including those based on physical cross-linking, with modulus in the upper kPa range need to be explored.

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