Recent Developments on Citric Acid Derived Biodegradable Elastomers

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Abstract: Biodegradable elastomers have recently found widespread application in many areas of biomedical engineering such as tissue engineering, drug delivery, and bioimaging. In particular, the recent developments in research have led to the creation of citric acid based polymers with enhanced mechanical properties, novel design strategies for crosslinking, nanoporous features, and unique photoluminescent capabilities. The present review will cover the recent patents involving citric acid derived biodegradable biomaterials within the field of biomedical engineering including poly (diol citrates) and their composites, crosslinked urethane-doped polyesters (CUPEs), poly (alkylene maleate citrates) (PAMCs), poly (xylitol-co-citrates) (PXC), and aliphatic biodegradable photoluminescent polymers (BPLPs). The synthesis, development, and applications of these novel polymers will be discussed along with the current trends and future developments in the biomaterials field.

Keywords: Biodegradable, elastomer, hydrogel, photocrosslinking, photoluminescence, citric acid, nanomedicine, tissue engineering, drug delivery, bioimaging.

1. INTRODUCTION

Biodegradable polymers have made a considerable impact in various fields of biomedical engineering including tissue engineering and drug delivery, where cell-seeded constructs are designed to replace damaged or diseased tissues [1, 2]. Ideally, tissue-engineered constructs should resemble the physical properties of the native tissues, which are mostly soft and elastic, and provide structural stability to the developing tissues [3]. In order to successfully engineer many of the native tissues, the resulting scaffold must be strong enough to withstand the mechanical demands asserted upon them once implanted inside the body, and be able to transfer mechanical stimuli to the newly developing tissue [4]. Unfortunately, the Food and Drug Administration (FDA) approved polylactones, poly (L-lactide) (PLLA), poly (glycolide) (PGA), and their copolymers (PLGA), are stiff and incompliant, which limit their use in many applications involving soft tissues [5-7]. The mechanical irritation resulting from the compliance mismatch between the scaffold and native tissue often leads to inflammation and scar formation, which ultimately prevents the implant from being effectively integrated with the surrounding tissue [8].

As a result, many groups have focused on the synthesis, characterization, and application of materials with a wide range of biodegradable and elastomeric properties [9-13]. Biodegradable elastomers are advantageous in that they can sustain and recover from multiple deformations without causing irritation to the surrounding tissue in a mechanically demanding environment [14-16]. Another advantage of an elastomeric scaffold is their ability to be used with mechanical conditioning regimens to promote improved tissue formation. By gradually transferring stress from the degrading synthetic matrix to the newly forming tissue, scaffolds with applied cyclic mechanical strains have been shown to increase collagen and elastin production in vascular smooth muscle cells [17, 18]. Numerous biodegradable elastomers have been developed for tissue engineering, and have found widespread use in the engineering of blood vessels, heart valves, nerves, cartilage, skin, bladder, and bone [19-25].

Among the materials, citric acid derived biodegradable elastomers (CABEs) have been shown to offer a wide range of controllable mechanical and degradation profiles along with surface affinities towards many cell types [26-28]. This new class of biomaterials is all synthesized with non-toxic monomers using simple and cost effective procedures. These materials all share one common monomer named citric acid, which is a non-toxic metabolic product of the Krebs cycle and has been approved by the Food and Drug Administration [29, 30]. For all CABE designs (Table **1**), citric acid is a versatile monomer that participates in pre-polymer formation through a simple polycondensation reaction while preserving pendant functionality for post-polymerization to produce a crosslinked polyester network with degradable ester bonds. Crosslinking confers elasticity to the polymers similar to the extracellular matrix, in which collagen and elastin are all crosslinked polymers [31]. In addition to the multifunctionality and biocompatibility of citric acid, the sodium form of citric acid, sodium citrate, is an anti-coagulant currently used in hospitals [32]. Thus, it is expected that CABEs may also possess suitable hemocompatibility for blood contacting applications.

CABEs have been applied in a wide variety of fields including vascular tissue engineering, orthopedics, medical device coatings, wound dressings, and drug delivery

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Table 1. Citric Acid Based Elastomers Full Names, Abbreviations, and Their Potential Applications.

Polymer	Abbreviation	Potential Applications
Poly (1,8-octanediol)	POC	Vascular tissue engineering Medical device coatings Drug delivery
Poly (1,8-octanediol)-hydroxyapatite	POC-HA	Orthopedic fixation devices Bone tissue engineering
Crosslinked urethane-doped polyester	CUPE	Tissue engineering Orthopedic applications
Poly (alkylene maleate citrate)	PAMC	Soft tissue engineering Drug delivery Wound healing/wound dressing
Poly (poly (ethylene glycol) maleate citrate)	PPEGMC	*In situ* tissue engineering Drug delivery Wound dressing
Poly (xylitol-co-citrate)	PXC	Tissue engineering Drug delivery
Biodegradable photoluminescent polymer Crosslinked biodegradable photoluminescent polymer Crosslinked urethane-doped biodegradable photoluminescent polymer Water soluble biodegradable photoluminescent polymer	BPLP CBPLP CUBPLP WPBLP	Tissue engineering, drug delivery, bioimaging probes, and biosensors

applications. More recent developments have also shown the ability of CABEs to be used as bioimaging materials for the in vivo fluorescent monitoring of tissue engineered scaffolds and in vitro cellular labeling. In this review, the focuses are placed on the recent advancements made involving CABEs, and the future status of these materials in biomedical engineering applications.

2. POLY (DIOL CITRATES)

2.1. Synthesis and Characterization of Poly (diol citrates)

In 2004, Yang *et al.* synthesized the first CABE, poly (diol citrate), a convenient and cost effective polycondensation reaction [4, 33]. As seen in Fig. (**1**), citric acid was used as a multifunctional monomer to react with different aliphatic diols ranging from 3-16 carbon chain lengths in a 1:1 molar ratio at 140°C under mechanical stirring to form a pre-polymer, which could be further post-polymerized into crosslinked polyester networks under various polycondensation conditions [34-36]. The resulting material has been shown to cover a wide range of mechanical properties, degradation profiles, and surface energies, which are all important in controlling the biological response to an implanted material [34].

By controlling the post-polymerization temperature and time, the elastomer's mechanical properties and degradation rates can be tuned to fit a wide range of tissue engineering applications. An increase in post-polymerization temperature and time resulted in a network with increased mechanical properties due to the increased crosslinking densities. As seen in Table **2**, the reported range of mechanical properties for this family of elastomers meet the specific needs for the engineering of various soft tissues including cartilage, blood vessels, and bladders. The preliminary biocompatibility evaluations showed that poly (diol citrates) supported the attachment and proliferation of human aortic smooth muscle cells and endothelial cells without any surface modifications

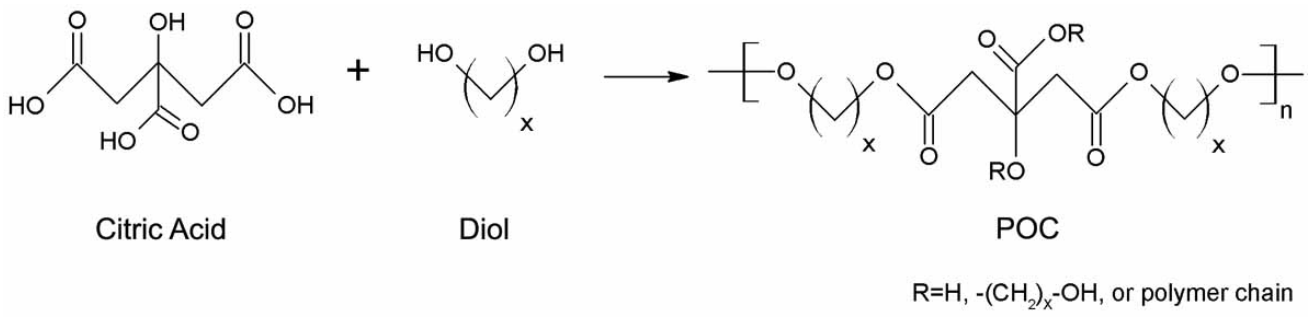

Fig. (1). Synthesis schematic of the poly (diol citrate) family.

Table 2. Characterization of Citric Acid Based Elastomers and Various Soft Tissues

Polymer	Tensile Strength (MPa)	Modulus (MPa)	Elongation (%)	Degradation Rate	Tested Cells and Animals	Ref.
POC	2.93-11.15	1.85-13.98	117-502	100% in 26 weeks [a]	HASMCs/HAECs Sprague-Dawley Rats	[26]
POC-HA	20-408	23-26	-	12% in 20 Weeks [b]	Human osteoblasts Rabbit	[28]
CUPE	14-37	2.2-32	217-309	15% in 8 weeks [c]	3T3 fibroblasts Sprague-Dawley Rats	[37]
BPLP and CBPLP	2.2-7.6	2.4-8.9	140-272	100% in 16 days and 31 weeks [d]	3T3 fibroblasts Nude mice	[27]
POMC	0.2-1.03	0.03-1.54	48-534	20-80% in 10 weeks[e]	3T3 fibroblasts Balb/C mice	[38]
PXC	-	0.0058±1.2	79.9±5.6	100% in 10 days [f]	Human fibroblasts Lewis Rat	[2]
Ulnar peripheral nerve	9.8-21.6	-	8-21	-	-	[39]
Human bladder	0.27±0.14	0.25±0.18	0.69±0.17	-	-	[40]
Human coronary artery	1.4-11.14	-	-	-	-	[41]
Bovine elastin	-	1.1	-	-	-	[42]
Smooth muscle relaxed	-	0.006	300	-	-	[43]
Human ACL	24-112	-	-	-	-	[44]
Human cartilage	3.7-10.5	-	-	-	-	[41]
Porcine lung	-	0.005	-	-	-	[45]

[a] For POC (80˚C, 2d) incubated in PBS (pH 7.4; 37°C).
[b] For POC-HA (65% HA) (80˚C, 3d, then 120˚C, 1d) incubated in PBS (pH 7.4; 37°C).
[c] For CUPE1.2 (80 ˚C, 2d) after 8 weeks of incubation in PBS.
[d] For BPLP-Cys0.2 and CBPLP-Cys0.8 (80˚C, 2d) incubated in PBS (pH 7.4; 37°C).
[e] For POMC at different citric acid/maleic acid ratios incubated in PBS (pH 7.4; 37°C).
[f] *in vivo* degradation.

[26]. Histological analysis of poly (1, 8-octanediol) (POC) films (a representative poly (diol citrate)) subcutaneously implanted in Sprague-Dawley rats confirmed the material biocompatibility. After 4 months of implantation, the inflammatory response and thickness of the fibrous capsule was smaller than the reported values of commonly used biodegradable polymers, poly (D,L-lactic-co-glycolide) (PLGA) [26, 34].

Since the mechanical properties of this new family of elastomers could be tuned to fit the needs of vascular tissue engineering, Yang. disclosed a recent patent on the fabrication of a novel biphasic scaffold for small diameter blood tissue engineering Fig. (**2**) [34]. These scaffolds consisted of a non-porous lumen and porous outer phase. The non-porous phase was designed to provide a continuous surface for the adhesion and proliferation of endothelial cells while maintaining the strength and elasticity of the construct. The outer porous phase was designed to facilitate the three-dimensional growth of smooth muscle cells. Burst pressures for this type of scaffold were as high as 2800 mmHg, which are similar to that of the native small diameter arteries [36].

Fig. (2). Soft and elastic poly (diol citrate) biphasic scaffold consisting of a non-porous lumen and porous outer phase for small diameter blood vessel tissue engineering.

The overall results suggest that this biphasic scaffold design using poly (diol citrates) is a viable strategy towards the engineering of small diameter blood vessels [46].

2.2. Modulating Host Response to Synthetic Vascular Grafts Via Poly (diol citrates)

The use of synthetic grafts such as polyethylene terephthalate (Dacron) or polytetrafluoroethylene (ePTFE) has been limited to large diameter (>6 mm) blood vessel applications in the treatment of atherosclerotic vascular disease. The surface of these synthetic grafts is innately thrombogenic, and their hydrophobicity limits endothelial formation, which causes early graft occlusion from thrombosis [32]. Yang *et al.* was the first group to investigate the use of POC to surface-engineer a functional elastic coating on ePTFE graft lumens without affecting the compliance of the graft. The rationale behind this design was to use POC as a substrate to interlock the newly formed endothelium and underlying ePTFE to improve the early thromboresistance and inhibit neointimal hyperplasia. As mentioned previously, sodium citrate, a salt form of citric acid, is often used as an anticoagulant in hospitals. Thus, the calcium-chelating properties could be used to reduce the thrombogenicity of a material.

Compared to control ePTFE grafts, the POC modification to ePTFE reduced thrombogenicity and promoted endothelialization. POC was able to significantly reduce both the clotting times of plasma and platelet activation, which displayed signs of the CABE's ability to enhance hemocompatibility. Porcine endothelial-like cells seeded onto POC modified ePTFE attached, proliferated, and were confluent after 10 days of culture, which suggested that the POC coating was conducive for endothelialization. When implanted in a porcine iliac artery, the modified grafts significantly showed improvements in hemocompatibility and inflammatory response over control ePTFE grafts [47]. This new material based approach was a major step in engineering a functional vascular graft for the replacement of small diameter blood vessels.

2.3. Poly (diol citrate) Composites

Previous studies have shown that the mechanical properties of an elastomer can be enhanced by adding a second component to the elastomeric phase of the polymer [41]. Webb. have demonstrated that the strength and stiffness of poly (diol citrates) can be increased without losing the material's elasticity by introducing a biodegradable polymeric nanophase into the network. This is the first report on the creation of a nanocomposite composed of a biodegradable micro and nanophase. The nanoparticles/nanostructure act as additional crosslink points to reinforce the polymer's network chains to increase the mechanical properties of the resulting material [41]. When PLLA or PLGA were incorporated as the nanophase into the poly (diol citrate) network, the tensile strength and modulus increased from 1.51 ± 0.08 to 3.54 ± 0.35MPa and 1.59 ± 0.13 to 17.73 ± 1.99MPa respectively when compared to poly (diol citrate) controls. Due to the enhanced mechanical properties and biocompatibility of the composite, the elastomer network has been targeted for applications including tissue patches and the engineering of cartilage and ligaments where increased strength is required.

Poly (diol citrates) composites with hydroxyapatite (HA) have also been used for bone tissue engineering. HA is a bioceramic that is naturally present in bone, and has been shown to improve the osteoconductivity of polymers [48]. In 2006, Qiu *et al.* was the first group to produce a bioceramic-elastomer composite based upon HA and poly (diol citrate), which showed mechanical properties comparable to native bone. The bioactive ceramic component was able to be incorporated up to 65% of the entire composite weight, and maximized the osteointegration while maintaining the elastomer's degradability [28]. The elastomer composite successfully induced surface mineralization after 15 days of incubation in simulated body fluid, and displayed favorable primary human osteoblast cell adhesion with no chronic inflammation after implantation in rabbits. Composites implanted into rat medial femoral condyle appeared to be well integrated with the surrounding cartilage along with mineralized chondrocytes located immediately adjacent to the implant, which suggests normal bone remodeling. The composites also showed good processability in the ability to be machined and molded into bone screws for bone fixation applications [35].

2.4. Nanoporous Poly (diol citrates)

Macroporous poly (diol citrates) scaffolds created by the traditional salt-leaching method have been well tested for tissue engineering applications. However, salt-leached synthetic scaffolds normally consist of a large amount of dead pores which are not suitable for cell infiltration and mass transport. Mathew *et al.* have developed an improved poly (diol citrates) scaffold fabrication technology by using a secondary porogen, poly (ethylene glycol) dimethyl ether (PEGDM) along with the sieved salts [49]. It was demonstrated that PEGDM may partially participate in the formation of a crosslinked poly (diol citrates) network, thus modulating the degradation and mechanical properties of the resulting poly (diol citrates) scaffolds. The majority of PEGDM was leached out resulting in nanoporous features on the scaffolds, which was expected to enhance the cell infiltration and mass transport.

A recent development by Hoshi. has broadened the scope for nanoporous poly (diol citrates) into the drug delivery field. Previous biodegradable polymer designs utilized in drug delivery systems are associated with inherent limitations including drug deactivation, and compliance mismatch between the biomaterial and native tissue [50]. Hoshi *et al.* demonstrated the potential of POC to be used as a biodegradable drug delivery system, which was capable of efficiently entrapping the drug under normal physiological conditions. In order to entrap the drug, a PEGDM nanoporogen was introduced into POC to encourage phase separation during the post-polymerization process. Using this novel strategy, POC substrates with pores on the order of hundreds of nanometers were fabricated with porosities of up to 86.8% [50]. Upon freeze-drying, the nanopore structures created from the PEGDM collapsed, which results in a construct with porosities of only 10%. This phenomenon

shows the potential to control the porosity and drug encapsulation through the drying process.

Nanoporous samples loaded with Dextran and freeze-dried to encapsulate the drug displayed a delayed burst release and slower cumulative release for up to 3 days when compared to nanoporous samples which had not been freeze-dried. The nanoporous POC constructs were shown to have degraded between 20-100% within a 2-week period after incubation in a phosphate buffered solution, and was modulated through the amount of PEGDM introduced into the polymer. Nanoporous POC films subcutaneously implanted into Sprague-Dawley rats showed no signs of an acute or chronic inflammatory response after 2-weeks of implantation. Thus, the biocompatibility and ability for drug encapsulation make nanoporous POC a promising tool for drug delivery and tissue engineering applications.

2.5. Fumarate-containing Poly (diol citrates)

Previous poly (diol citrates) could only be crosslinked by a thermal crosslinking mechanism. In addition, the polycondensation-based elastomers are unable to be rapidly crosslinked into complex geometries, a major limitation affecting the processability of these materials [51]. In 2009 Zhao *et al.* were able to increase the range of mechanical properties for POC by incorporating an additional mode of crosslinking. Acrylate or fumarate moieties were introduced to provide a secondary crosslink network through free radical polymerization. This extra crosslinking method might also show POC's potential to be rapidly processed into complex geometries.

By incorporating the secondary crosslinks within the polyester network, the initial modulus, peak stress, and elongation at break increased to 38 MPa, 10 MPa, and 260%, respectively when compared to POC control materials. Although the crosslinks created during the radical polymerization are not readily degradable, the ester bonds, which connect the free radical crosslinks to the rest of the polyester network, are hydrolyzable. Polymer samples were shown to have degraded 20% and 30% after two months of incubation in phosphate buffered solution for polymers created from acrylated pre-polymers and fumarate containing pre-polymers, respectively.

3. CROSSLINKED URETHANE-DOPED POLYESTERS (CUPES)

Although poly (diol citrates) have shown great potential in the field of tissue engineering, the design and development of a soft, strong, and completely elastic (100% recovery from deformation) material was still a challenge. Polymers used for tissue engineering lose a significant amount of mechanical strength when fabricated into porous scaffolds. For example, poly (diol citrate) underwent a significant loss in peak stress from 2.93 ± 0.09MPa (film) to 0.3 ± 0.1 (scaffold) upon pore introduction [46]. In 2008, Dey *et al.* successfully combined the advantages of a fully elastic crosslinked polyester network with the strength of linear polyurethanes in a recent patent disclosure. By doping urethane bonds into the poly (diol citrate) polyester network, a new generation of CABEs, named crosslinked urethane-doped polyesters (CUPEs) was introduced with improved mechanical strength Fig. (**3**). CUPE pre-polymers were created by first reacting a 3 wt.-% pre-POC in 1,4-dioxane solution with hexamethylene diisocyanate (HDI). Various molar ratios of the HDI/pre-POC (0.9, 1.2, and 1.5) were reacted at 55°C under mechanical stirring to create a group of elastomers with very strong mechanical properties. Similar to poly (diol citrates), citric acid provides pendant

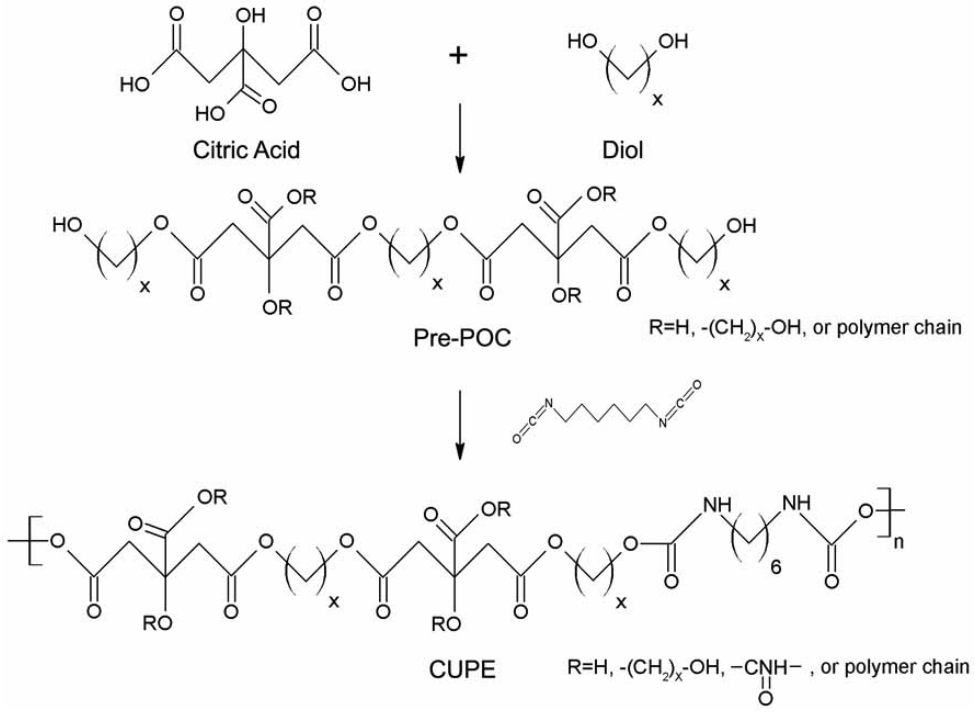

Fig. (3). Representative crosslinked urethane-doped polyester (CUPE) synthesis schematic.

carboxylic and hydroxyl functionality for ester bond formation during the post-polymerization process to produce a degradable crosslinked polyester network. The amount of crosslinking between the polymer chains was controlled through the post-polymerization temperature and duration. The doping of urethane bonds into the polyester acted as a chain extender and enhanced hydrogen bonding within the network to produce elastomers with peak stresses as high as 41.07 ± 6.85MPa while still maintaining over 200% elongation at break [37]. Amazingly, a simple chemical modification to the previous poly (diol citrate) resulted in over a 10-fold increase in mechanical strength.

CUPE polymers could be tuned to meet a variety of needs by varying the length of the diol used in the pre-polymer synthesis, diisocyanate ratio, and post-polymerization conditions [52]. Preliminary cytocompatibility results showed that 3T3 fibroblast and smooth muscle cells were able to adhere and proliferate onto CUPE surfaces with growth rates comparable to PLLA. Soft and elastic three-dimensional porous sheets (150μm thick) could be fabricated from a simple thermally induced phase separation technique (TIPS). Unlike the previous poly (diol citrates), the higher molecular weights and the non-sticky nature of the CUPE pre-polymers allows the use of other scaffold fabrications techniques such as TIPS and electrospinning [37]. As seen in Fig. (**4**), the cross section of a CUPE scaffold sheet shows the highly porous structure produced from the TIPS technology. The thin scaffold sheets allowed for even seeding, growth, and distribution of 3T3 fibroblasts. Future work involving this family of CABEs will be focused on characterizing the material using different carbon length diols, and the fabrication of novel scaffolds for small diameter blood tissue engineering.

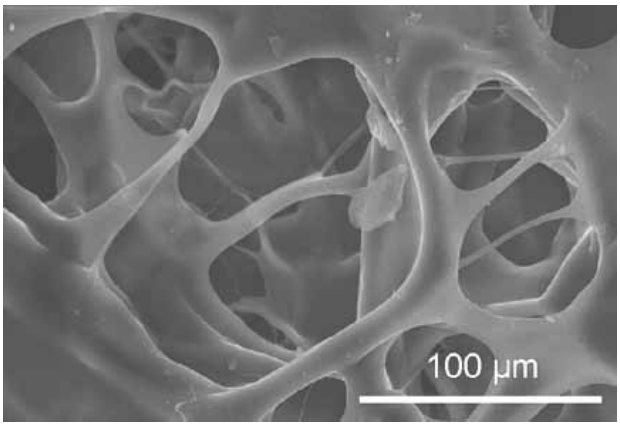

Fig. (4). Scanning electron micrograph of a highly porous crosslinked urethane-doped polyester (CUPE) scaffold cross section produced from a thermally induced phase separation technique.

4. POLY (ALKYLENE MALEATE CITRATES) PAMC

4.1. Synthesis and Characterization of Poly (alkylene maleate citrates)

Photocrosslinkable biodegradable materials have recently attracted increased attention in tissue engineering, drug delivery, and wound care applications [53, 54]. Recently, Yang *et al.* registered a type of CABEs referred to as poly (alkylene maleate citrates) (PAMCs) based on previous poly (diol citrates), which can be quickly crosslinked into a thermoset elastomer through a dual crosslinking mechanism (DCM) [38]. Similar to the work done by Zhao *et al.*, PAMCs were created by introducing a vinyl-containing moiety into the pre-polymer network [51]. These polymers can be crosslinked either by photo-crosslinking, thermo-crosslinking, or both mechanisms. A random polycondensation reaction between C3-C16 diols, citric acid, and a vinyl–containing monomer (maleic acid or maleic anhydride) was carried out at 140°C for 4 hours to form a pre-polymer of PAMC with degradable ester bonds and vinyl functionality incorporated into the polymer backbone Fig. (**5**).

The DCM allows the polymer to be quickly C-C crosslinked through redox or ultraviolet systems to preserve valuable pendant carboxylic and hydroxyl groups from citric acid in the bulk of the material for potential bioconjugation. PAMC networks crosslinked through this route also show a pH dependent swelling capability, which could be very useful in targeted drug delivery applications. The free radical crosslinking method can also be combined with an additional thermo-crosslinking mechanism to further crosslink the network in order to fine-tune the mechanical properties and degradation profiles to meet the variety of soft tissue engineering applications.

As seen in Table 2, the PAMC family of elastomers has a wide range of mechanical properties (Young's Modulus of 0.05–1.8MPa) and elasticity (elongation of 45–450%) that can be modulated through different monomer ratios, photoinitiator concentration, polymer concentration while crosslinking, and the DCM. Cells seeded onto PAMC surfaces and encapsulated in PAMC gels exhibited normal spread morphologies. Preliminary biocompatibility results show a decline in the inflammatory reaction and reduction in capsule thickness over a 4-week period. No tissue necrosis was found throughout the animal studies.

4.2. Potential Applications for Poly (alkylene maleate citrates)

PAMCs can be quickly polymerized within 3 minutes to seal any defects on the immediate surface. This PAMC property shows its potential in wound dressing and tissue sealant applications. Furthermore, pre-poly (poly (ethylene glycol) maleate citrate) (PPEGMC) is another member of the PAMC family that is soluble in water, and can be used as an injectable crosslinkable polymer for *in situ* tissue engineering and drug delivery applications. PAMC polymers can also be combined with Micro–Electro–Mechanical Systems (MEMS) technologies to produce novel substrates for tissue engineering. Creating tissue constructs using three-dimensional (3D) scaffolds has been a heavily researched area [55]. However, creating constructs that provide adequate nutrient and oxygen transport to cells deeply embedded within the constructs has been a significant challenge [56]. The development of an established vasculature system to provide oxygen, nutrients, and waste removal is critical in the survival of tissue engineered organs [57].

To address the above concerns, our lab has attempted to construct microchannels directly onto 3D porous scaffolds.

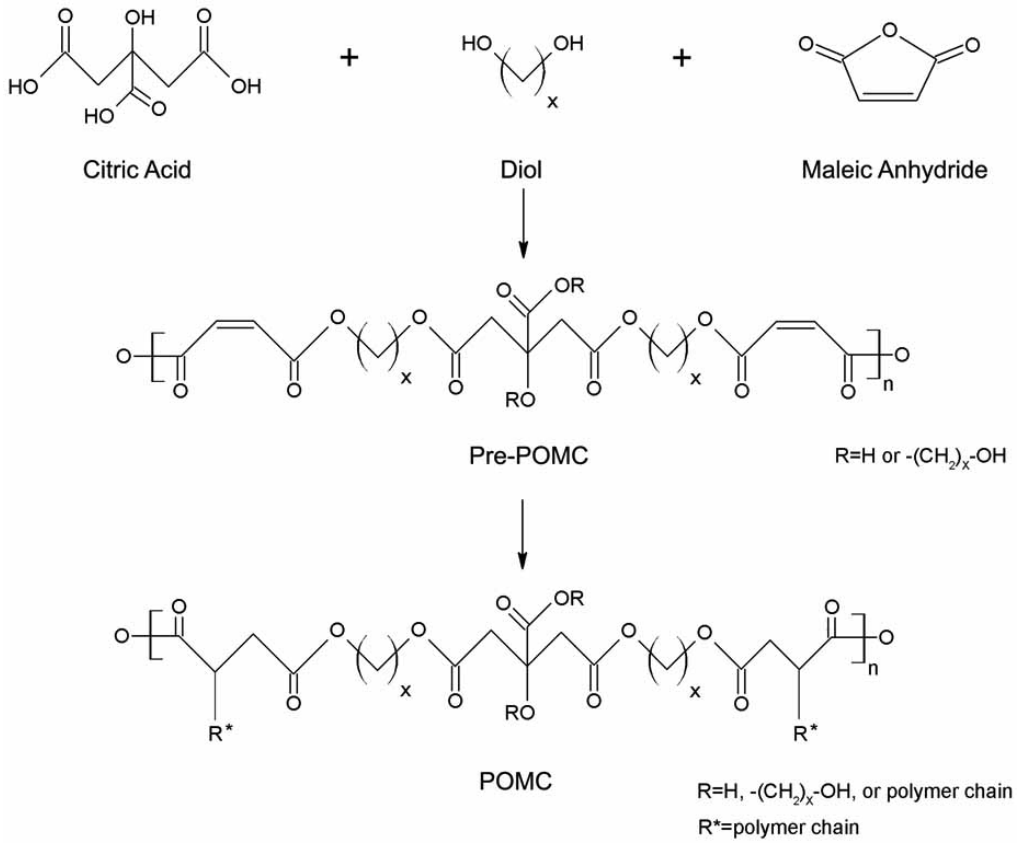

Fig. (5). Representative poly (alkylene maleate citrate), poly (octamethylene maleate anhydride citrate) synthesis schematic.

As seen in Fig. (**6**), a microchannel-patterned PAMC scaffold was fabricated in our lab. Briefly, a pre-PAMC solution was cast onto the polydimethylsiloxane microchannel mold, and exposed to ultraviolet irradiation to crosslink the polymer. Once the polymer was formed in the microchannels, the particulate leaching method was used to fabricate porous PAMC scaffolds onto the microchannels. Stacking such scaffolds may form 3D porous scaffolds featured with embedded microchannel networks within the scaffolds. These embedded microchannels are expected to provide physical guidance for vascularizing critical-sized tissue engineering constructs. The interface of the channel phase and porous phase can be made into a thin and porous structure to permit the communication between cells on both phases. This is the first time that a microchannel structure was incorporated on 3D porous structures.

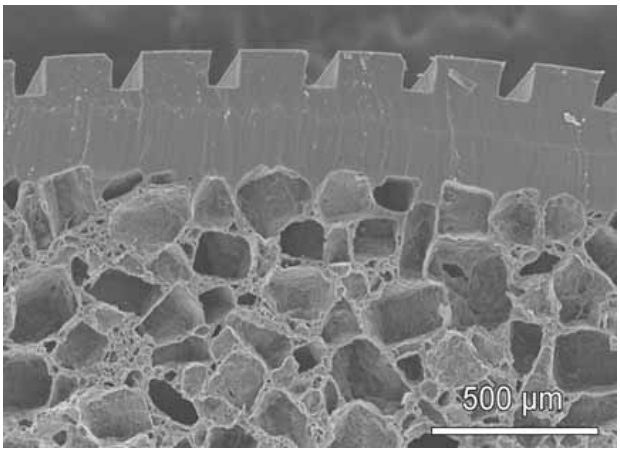

Fig. (6). Scanning electron microscope picture of poly (alkylene maleate citrate) scaffold cross section using microelectromechanical (MEMS) technology. Soft and elastic microchannels have been incorporated on traditional particulate leached scaffolds.

5. POLY (XYLITOL-CO-CITRATE) (PXC)

Bruggeman and coworkers first reported a polymer based on xylitol and citric acid referred to as poly (xylitol-co-citrate) **(**PXC) [2]. The rationale behind this design was to create a biologically relevant water-soluble pre-polymer using non-toxic FDA approved monomers endogenous to the human metabolic system. PXC was synthesized by reacting xylitol with citric acid at 150°C and 40mTorr for 1-12 hours. By using citric acid as a multifunctional monomer, PXC is able to form a randomly crosslinked network resulting in a polymer with elastic behavior. The pendant functionalities of xylitol were further modified with an acrylate group donated by methacrylic anhydride Fig. (**7**). It was claimed that through the use of xylitol, a metabolic intermediate in the mammalian carbohydrate, and citric acid, PXC should display good biocompatibility.

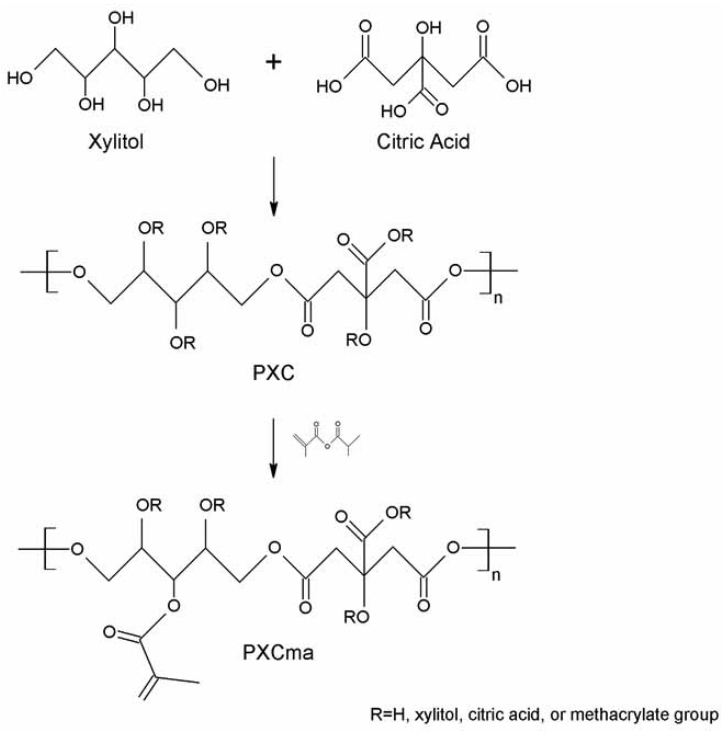

Fig. (7). Synthesis schematic for poly (xylitol-co-citrate) (PXC).

PXC displayed a compressive modulus of 5.84 ± 1.15kPa with a maximum compressive strain of 79.9 ± 5.6%. During the cyclic testing of this material, minimum hysteresis was reported confirming the limited deformability under cyclic strain. The targeted application of this polymer is the field of tissue engineering and drug delivery. The preliminary biocompatibility evaluations showed that this hydrogel does not support cell attachment, however, its pre-polymer chains are cytocompatible. Once inside the body, PXC degrades completely within two weeks and shows a comparable host response with PLGA. In general, this hydrogel is a promising candidate as a biomaterial for tissue engineering and drug delivery.

6. BIODEGRADABLE ALIPHATIC PHOTOLUMINESCENT POLYMERS (BPLP)

Biodegradable fluorescent polymers have attracted much attention in drug delivery and tissue engineering. However, in most of the published studies, biodegradable fluorescent polymers are made by either conjugating or encapsulating some organic dye or quantum dots (QDs) with the biodegradable polymers such as cyanine dye conjugated PLGA nanoparticles, rhodamine encapsulated PLA nanoparticles, porphyrin membrane-loaded polymer vesicles, or inorganic quantum dots encapsulated within polymers [58-62].

Yang *et al.* has recently discovered a family of novel aliphatic biodegradable photoluminescent polymers, referred to as BPLPs [27]. Unlike traditional non-degradable aromatic fluorescent polymers used in the lighting industry, BPLPs are aliphatic degradable oligomers synthesized using biocompatible monomers including citric acid, aliphatic diols, and different amino acids a very simple and cost-effective polycondensation reaction. BPLPs can be further polymerized into elastomeric crosslinked aliphatic biodegradable photoluminescent polymers (CBPLPs). These polymers offer advantages over the traditional fluorescent organic dyes, inorganic quantum dots, and non-degradable fluorescent polymers in terms of their excellent cytocompatibility, controlled degradability, and tunable photoluminescent properties with fluorescence emissions up to 725nm within the known BPLPs.

As seen in Fig. (**8**), the synthesis of BPLPs is very simple and is based upon the previously published poly (diol citrates). Briefly, one of the twenty (L-) amino acids is reacted with citric acid and aliphatic diols at 140°C to prepare BPLPs such as BPLP-cysteine (BPLP-Cys). The various forms of BPLPs (polymer solution, films, scaffold, and nanoparticles) have been shown to emit strong fluorescence. Fig. (**9**) Shows that only BPLP-Cys emits strong fluorescence indirectly demonstrating that BPLP-cys differs from the POC polymer (the precursor of BPLP). The fluorescent emission wavelength can be tuned by adding different amino acids to POC for BPLP synthesis. All 20 amino acids have been used to synthesize a family of BPLPs, which emit tunable fluorescence color from blue to red with a quantum yield up to 62.3%.

To test the potential of BPLP nanoparticles and CBPLP scaffolds for bioimaging purposes, BPLP-ser nanoparticles were injected subcutaneously into the back of nude mice. As shown in Fig. (**10**), the images of BPLP-ser porous scaffolds emit a strong fluorescence. Strong fluorescence was also emitted from the BPLP nanoparticles implanted in the subcutaneous cavities. The findings from the bioimaging studies lend strong support that BPLP nanoparticles and CBPLP scaffolds can be fluorescence-imaged, and the signals can be quantitatively analyzed and potentially for

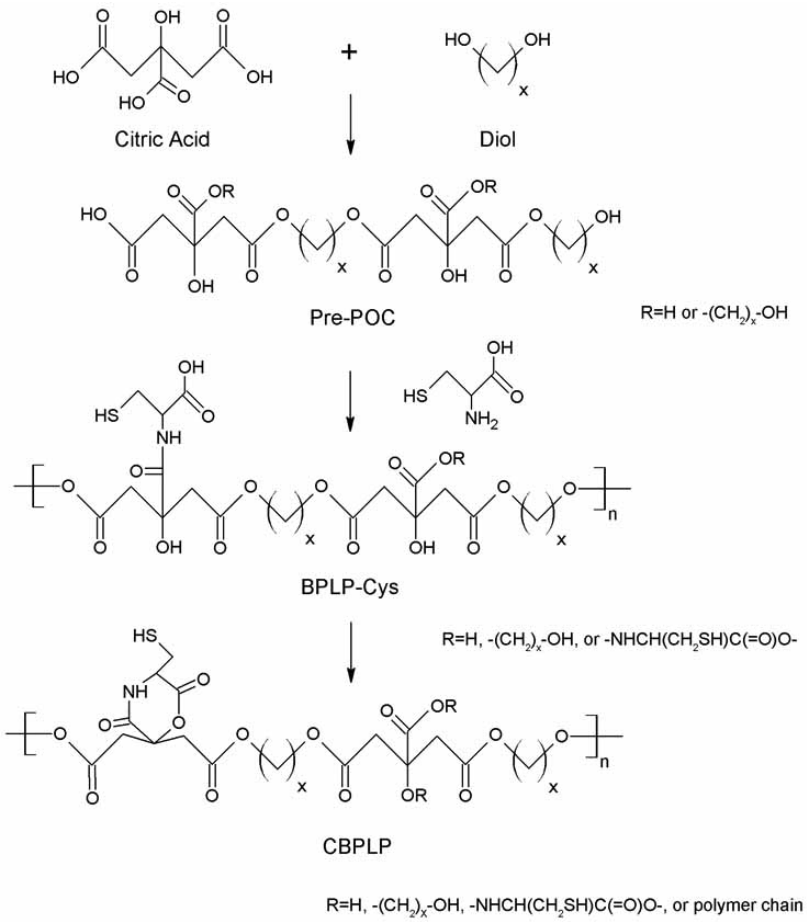

Fig. (8). Representative synthesis schematic of biodegradable aliphatic photoluminescent polymer reacted with L-cysteine (BPLP-cys).

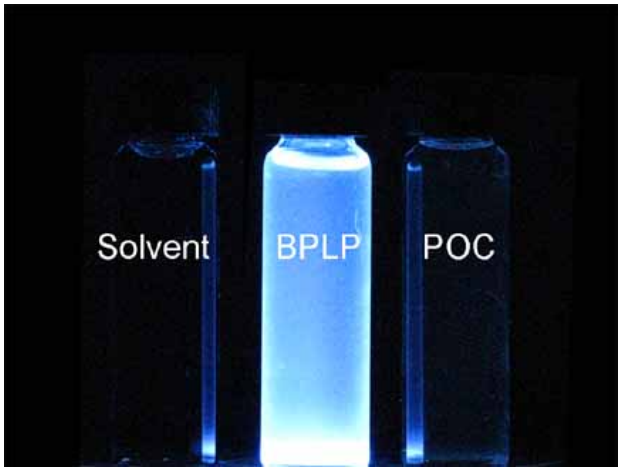

Fig. (9). Photograph of 1, 4-dioxane (left), biodegradable aliphatic photoluminescent polymer (BPLP-Cys) (center), and POC (right) in the presence of an ultraviolet light source.

cellular/tissue/scaffold bioimaging [63]. The development of BPLPs may address the urgent needs in nanomedicine for biodegradable theranostic biomaterials, which may deliver imaging agents and therapeutic probes in a single setting without using any inorganic imaging agents.

Based on the previous work on CUPE, PAMC, and BPLP, Yang *et al.* have worked on expanding the family of biodegradable fluorescent polymers. In a recent patent application, Yang *et al.* have synthesized mechanically strong crosslinked urethane-doped BPLPs (CUBPLPs),

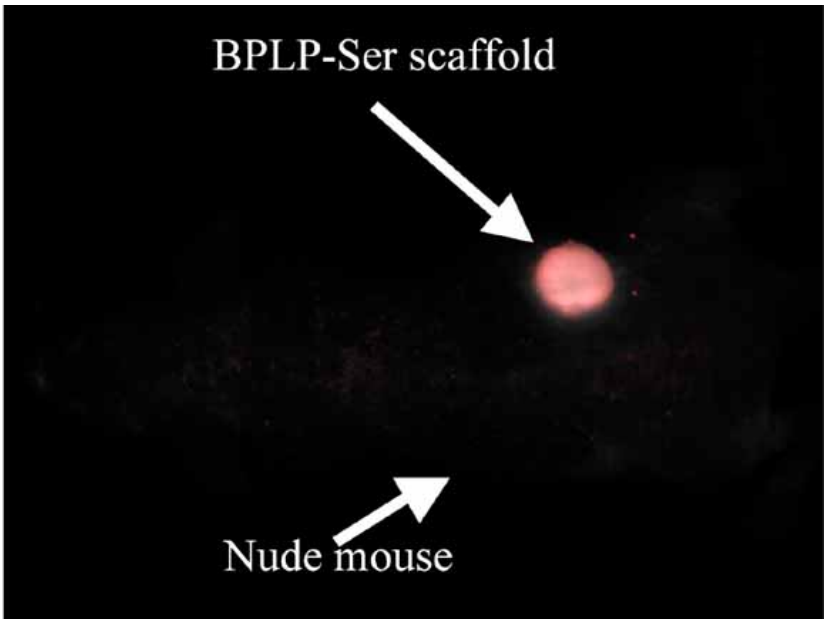

Fig. (10). Biodegradable aliphatic photoluminescent polymer scaffold implanted in nude mouse.

photocrosslinkable BPLPs (PBPLPs), and water soluble BPLPs (WBPLPs) to expand the choices of biodegradable photoluminescent polymers [63].

7. CURRENT & FUTURE DEVELOPMENTS

Since many of the tissues in the body are soft and elastic, the development of biodegradable elastomers has become increasingly important for tissue engineering soft tissues such as skin, blood vessels, tendons, ligaments, cartilage, and bladders. Soft and elastic scaffolds fabricated from biodegradable elastomeric materials not only provide a substrate for cells to adhere and proliferate, but also minimize the compliance mismatch between the implant and the surrounding tissues. As more stringent material requirements in personalized tissue regeneration are made, the design, synthesis, and potential applications of today's biodegradable elastomers will continue to evolve.

Citric acid derived biodegradable elastomers have shown great potential in meeting the requirements for soft tissue engineering. A key feature to understand is that citric acid acts as a robust multifunctional monomer to provide valuable pendant functionality to give the above listed polymers their unique advantages over existing biomaterials. Citric acid is mainly used to participate in the ester-crosslink formation in the biomaterials, but also enhances hemocompatibility, balances the hydrophilicity of the polymer network, provides hydrogen bonding, and additional binding sites for bioconjugation to confer additional functionality such as optical properties.

The future work of these materials will be focused on their biomedical applications. Poly (diol citrates) and their composites have shown promise in vascular graft coatings, orthopedic devices, and drug delivery. More intense animal studies for such applications are underway. CUPEs have addressed the challenges of developing soft, elastic, but strong biodegradable elastomers. Therefore, CUPEs enable a scaffold-sheet tissue engineering design in which soft, elastic, and thin CUPE scaffold sheets can be seeded with cells and stacked to form 3D tissue constructs for immediate implantations. The 2D cell-sheet tissue engineering approach has promised a revolution in tissue engineering [64-66]. However, cells grown in a 2D environment may lose cell characteristic or functions. In addition, 2D cell sheets are fragile and thus hard to handle [66, 67]. 3D scaffold sheet tissue engineering provides a 3D environment for cells to attach, grow, and differentiate. 3D scaffold sheets are soft, elastic, and strong allowing for an immediate implantation after cell seeding. Therefore, the 3D scaffold-sheet tissue engineering design may be the next direction for tissue construction in tissue engineering.

For certain tissue engineering applications, it is desirable to use *in situ* crosslinkable biodegradable materials as injectable scaffolds for tissue regeneration through a minimally invasive delivery method. Injectable biomaterials can potentially fill irregular defects by taking the shape of the cavity in which they are placed, thus avoiding the need for toxic solvents and patient specific scaffold prefabrication. Since many of the CABEs listed previously can be made into water-soluble polymers, such as water-soluble PAMCs and BPLPs, future efforts will be placed on utilizing those polymers for *in situ* tissue engineering applications such as wound dressings and bone fillers.

The unique photoluminescent polymers, BPLPs and CBPLPs also hold promise in nanomedicine and tissue engineering. An emerging field of nanomedicine is theranostic nanomedicine in which nanoparticles should be able to deliver therapeutic drugs and bioimaging probes in a single setting [68]. BPLP nanoparticles may innovate the field as BPLP nanoparticles are fully degradable and may deliver therapeutic drugs/ fluorescent bioimaging probes without using any toxic inorganic imaging agents such as quantum dots. CBPLPs also hold great promise in tissue engineering. The photoluminescent CBPLP scaffolds may allow a non-

invasive monitoring of scaffold degradation *in situ* without histological analysis, which is a challenge that has not been addressed. Understanding *in situ* real-time scaffold degradation is critical for biomaterial custom-design to meet the versatile needs of tissue engineering.

In addition to the engineering of tissues, there has been a lack of progress in fully understanding the cell, material, and host tissue interactions. Despite the recognized importance of the mechanical properties of tissue engineering scaffolds on tissue development, there has been a dearth on fundamental understanding on how the mechanical properties of the scaffold affect the inflammatory response of the host and the tissue/graft integration.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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