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Biocompatibility of polysebacic anhydride microparticles with chondrocytes in engineered cartilage

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Abstract

One of main challenges in developing clinically relevant engineered cartilage is overcoming limited nutrient diffusion due to progressive elaboration of extracellular matrix at the periphery of the construct. Macro-channels have been used to decrease the nutrient path-length; however, the channels become occluded with matrix within weeks in culture, reducing nutrient diffusion. Alternatively, microparticles can be imbedded throughout the scaffold to provide localized nutrient delivery. In this study, we evaluated biocompatibility of polysebacic anhydride (PSA) polymers and the effectiveness of PSA-based microparticles for short-term delivery of nutrients in engineered cartilage. PSA-based microparticles were biocompatible with juvenile bovine chondrocytes for concentrations up to 2mg/mL; however, cytotoxicity was observed at 20mg/mL. Cytotoxicity at high concentrations is likely due to intracellular accumulation of PSA degradation products and resulting lipotoxicity. Cytotoxicity of PSA was partially reversed in the presence of bovine serum albumin. In conclusion, the findings from this study demonstrate concentration-dependent biocompatibility of PSA-based microparticles and potential application as a nutrient delivery vehicle that can be imbedded in scaffolds for tissue engineering.

Keywords

Articular cartilage; Polysebacic anhydride; Microparticles; Delivery; Lipotoxicity; Hydrogel

1. Introduction

Articular cartilage, the connective tissue of diarthrodial joints, absorbs and distributes large compressive stresses placed on the joint, and enables low friction sliding. Treatment of diseased or damaged articular cartilage is challenging due to its avascular nature and poor self-repair capability [1,2]. Cartilage tissue-engineering techniques that encapsulate

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chondrocytes within a three-dimensional scaffold have been successful in promoting extracellular matrix deposition *in vitro* [3–5]. However, nutrient diffusion into the engineered tissue decreases over time due to the elaborated extracellular matrix on scaffold periphery. As a result, compressive mechanical and biochemical properties are lower in the center of the scaffold than the periphery [6]. One approach to overcome limited nutrient diffusion is to decrease the diffusion path length through macro-channels [6] or localized delivery of nutrients throughout the scaffold. Improved nutrient diffusion from macro-channels can be limited due to cells on the channel periphery depositing extracellular matrix into the channel and occluding the channel within a few weeks in culture [6]. In this study, we use nutrients-encapsulated microparticles to deliver nutrients throughout the scaffold.

There are several current and emerging applications that have used biocompatible polymers to fabricate delivery vehicles for drugs, genes, and vaccines [7,8]. One challenging factor of using delivery vehicles for nutrient distribution is the ability to accurately control the release profile and to provide uniform distribution of delivered nutrients. For example, transforming growth factor-β3 (TGFβ3), which is crucial for cartilage growth and development, must be delivered uniformly during the first two weeks in culture [9,10]. Therefore, an ideal polymeric delivery device should be biocompatible and exhibit a surface erosion profile with fast release kinetics (i.e., order of weeks).

Polyanhydrides are a class of biodegradable and biocompatible polymers that are used for sustained delivery of bioactive agents [11–13]. They have several advantages, including surface erosion properties and tunable degradation rates suitable for short-term release profiles [14,15]. Previous studies have used polyanhydrides to deliver vaccines, adjuvants, and proteins, and demonstrated that the immunogenicity of these actives is preserved [16–19]. Moreover, polyanhydride-based wafers are approved by the Federal Drug Administration (FDA) for drug delivery to treat brain tumors [20].

Polymer degradation rate is highly dependent on molecular weight, hydrophobicity, pH, crystallinity, porosity, and surface area of the polymeric device [21–27]. Biocompatible polyanhydrides such as poly(sebacic anhydride) (PSA) prepared from sebacic acid can degrade at a faster rate than other biocompatible polymers (e.g., poly(esters)) [14,15,28]. Copolymerization of PSA with an aromatic anhydride (e.g., poly(carboxyphenoxy propane-co-sebacic anhydride), P(CPP-SA)) decreases degradation rate, compared to PSA-alone [14]. Polyanhydrides and their degradation products are considered non-cytotoxic [13,29–35], non-mutagenic [36], non-carcinogenic [13], and are extensively metabolized by rat brain tissue [37,38]. In particular, 80% of the sebacic acid was metabolized and eliminated as CO_2 [37,38]. Other cells, such as aorta epithelial osteoblast-like and smooth muscle cells, also did not show cytotoxic effects due to polyanhydrides [13,39]. However, recent work suggests that polyanhydrides are biocompatible for J774 macrophages ($0.5 - 1.25 \times 10^6$ cells/mL) for polymer concentrations up to 2.8 mg/mL, above which cytotoxicity was observed [40].

For nutrient delivery applications in cartilage tissue engineering, in situ release of sufficient bioactive molecules such as vitamin C would require a PSA microparticle concentration in excess of 50 mg/mL (see supplementary information). This necessary concentration (i.e.,

>50 mg/mL) for delivery of an appropriate concentration of nutrients throughout engineered cartilage may affect biocompatibility of polymeric microparticles due to accumulation of degradation by-products. Currently, there is no data in the literature that evaluates the biocompatibility and cytotoxicity of PSA with chondrocytes. Furthermore, there are no studies exploring the potential mechanisms of cytotoxicity or mitigation strategies. Without answering these questions, the utility of polyanhydrides (in particular PSA) as a short-term delivery device in articular cartilage tissue engineering will be very limited.

Therefore, the objectives of this study are to evaluate biocompatibility of PSA polymer with chondrocytes and to evaluate PSA-based microparticles as nutrients delivery device when embedded in agarose hydrogels for cartilage tissue engineering. Specifically, we found PSA polymer to be nontoxic to chondrocytes at or below 2 mg/mL PSA, above which cytotoxicity was observed. The mechanism of cytotoxicity at higher concentrations is deduced to be due to lipotoxicity because of structural similarities of PSA degradation products with fatty acids. Several mitigation strategies for PSA were tested and bovine serum albumin (BSA) treatment was found to be effective. The results from this study demonstrate potential use of PSA-microparticles with BSA protection as a nutrient or drug delivery vehicle in engineered cartilage.

2. Materials and methods

Chemicals required for synthesis of polymeric micro-particles were of analysis or higher grade: Sebacic acid (Alfa Aesar, Ward Hill, MA), methylene chloride (Acros Organics, New Jersey–US), poly(vinyl) alcohol (MP Biomedicals, Santa Ana, CA) petroleum ether (Fisher Scientific, New Jersey–US), acetic anhydride, ethyl ether, and amitriptyline hydrochloride from (Sigma Aldrich, St. Louis, MO). Aqueous solutions were made from Millipore™ water (with resistivity of ∼18.1 MΩ cm).

2.1. Synthesis and characterization of PSA polymer

The poly(sebacic anhydride) (PSA) was synthesized using sebacic acid as a monomer by melt condensation method [12]. Recrystallized sebacic acid (5 mg) was mixed with 50 mL of acetic anhydride and stirred for 20min at 40 °C until a clear solution was obtained. The excess acetic anhydride, after the de-hydration and acetylation of the diacid groups of sebacic acid monomer, was removed by increasing the temperature to 70–120 °C under vacuum. The precursor sebacic anhydride molecule was polymerized by increasing and maintaining temperature at 150 °C with constant stirring for 2 h. The final PSA product was dissolved in a minimum amount of methylene chloride and recrystallized using a large excess of ethyl ether and petroleum ether. The resulting suspension was centrifuged, dried at room temperature under vacuum, and stored at −20 °C. The chemical identity of the polymer was characterized using nuclear magnetic resonance (NMR) and vibrational spectroscopies which confirmed successful synthesis of the PSA polymer (see Supplementary information).

2.2. Fabrication of PSA microparticles and encapsulation of bioactive molecules

Fabrication of PSA microparticles with encapsulated nutrients was performed using a double emulsion method (water/oil/water) [41]. Methylene chloride was used as an oil phase and

poly(vinyl) alcohol (PVA) as an emulsifier at the interface between the external water and oil phases. An evaporative process was used to eliminate methylene chloride leaving behind the polymer and encapsulated bioactives in the internal water phase. Alternatively, bioactive solids were encapsulated by using them as the internal phase (solid/oil/water) instead of their aqueous solution. In particular, 100 mg of PSA was dissolved in the 1 mL of methylene chloride and the bioactive internal phase, either as aqueous solution or solid phase, was emulsified/dispersed by ultra-sonication and homogenization for 60 s. A typical internal phase composition is 5–50% by weight of the encapsulating polymer [41–43]. Therefore, we set the internal phase at 50% by weight of PSA (i.e. 50 μL of aqueous bioactive to 100mg of PSA) to maximize encapsulation. This water/oil emulsion was added drop-wise to 2 mL of the external phase consisting of aqueous solution of emulsifier (PVA) at 1 wt.% under continuous sonication and homogenization for 120 s. The obtained double emulsion was then diluted with 20 mL aqueous solution of 0.5% PVA and stirred vigorously for 4 h to evaporate the oil-phase (methylene chloride). A dense layer of polymer thus encapsulates the internal bioactive-phase forming microparticles. The encapsulated-microparticles suspension was centrifuged and washed with water three times. The precipitates were lyophilized and stored at −20 °C until use. The bioactive internal phases that are encapsulated include an aqueous solution of L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate. The microparticles intended for the delivery in the tissue constructs were fabricated under sterile conditions. Both the internal and external water phases in the double emulsion, as well as water in all stages of fabrication procedure, were replaced with Dulbecco's modified essential medium (DMEM) solution.

Scanning electron microscopy (SEM) was used to characterize size and morphology of PSA microparticles. The microparticles were deposited on a carbon substrate and coated with a 2 nm coating of Au-Pd and SEM images were obtained with a voltage setting of 0.8 kV and current at 20 mA (4700-Hitachi SEM, Roslyn Heights, NY).

Previous work on release kinetics from nanogel particles demonstrated that the release profile of amitriptyline, a tricyclic anti-depressant drug, was easily monitored by ultraviolet-light absorption (UV–light; wavelength = 239 nm) [44,45]. While amitriptyline has limited applications for cartilage tissue engineering applications, ascorbic acid oxidizes easily in the presence of water and dissolved oxygen, making it difficult to evaluate its release kinetics. Therefore, we evaluated the release kinetics of PSA microparticles by encapsulating microparticles with amitripty-line. Amitriptyline (15 mg) was dissolved in a phosphate buffered saline solution (PBS; 150 μL) and was encapsulated in PSA (200 mg) using double emulsion, as described above. Amitriptyline encapsulated PSA microparticles (175 mg) were resuspended in 10 mL of PBS. To measure the release concentration of amitriptyline from PSA microparticles, the suspension was centrifuged (7000 rpm for 5 min) and 5 mL of the supernatant was extracted once a week for three weeks. Fresh PBS (5 mL) was added at each time point to maintain the total volume. Amitriptyline standards were made in PBS (range 0 – 0.025 mg/mL) and used to determine the amount of amitriptyline released to the supernatant.

2.3. Tissue-engineering studies

Knee joints from bovine calves were acquired from a local abattoir. Articular cartilage was harvested aseptically and digested overnight in collagenase media (Type 4, Worthington Biochemical Corp. Lakewood, NJ). Chondrocytes were passaged in DMEM with 10% serum and a growth factor cocktail (0.5 ng/mL bFGF, 0.5 ng/mL PDGF, 1.0 ng/mL TGFβ1) [4]. Once cells reached confluence, they were removed from the culture dish by trypsinization.

Dosage-dependent biocompatibility was evaluated by encapsulating L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate (stabilized-vitamin C) in PSA microparticles. PSA microparticles were incorporated into a 4% weight/volume (w/v) agarose solution (40 °C, Sigma) at varying weight/volume percentages (0.2%, 2%, 3%, and 10% by w/v; 2, 20, and 100 mg/mL, respectively). Passaged cells (concentration of 60×10^6 cells/mL) were encapsulated in the agarose mixture for a final concentration of 30×10^6 cell/mL in 2% w/v agarose with PSA-microparticles. Each experimental group concentration was cast and studied in separate batches and thus had its own control without PSA microparticles.

Cylindrical constructs were cored from the agarose slab (diameter = 4 mm, thickness = 2.34 mm) and cultured in chemically defined medium (DMEM with 0.1uM dexamethasone, 40 mg/mL L-proline, 50 μg/mL ascorbate 2-phosphate, 100 mg/mL sodium pyruvate, 1× ITS +premix, 100 U/mL penicillin, and 100 mg/mL streptomycin and amphotericin B (Invitrogen Co., Carlsbad, CA)). The medium was changed every three days and supplemented with 10 ng/mL of TGFβ3 during the first 14 days in culture. Cell viability was assessed using a live/dead cytotoxicity kit (Molecular Probes, Invitrogen), where living cells are stained with calcein-AM and dead cells are stained with ethidium bromide.

The efficacy of bovine serum albumin (BSA) as mitigation agent for cytotoxicity of PSA polymer (not microparticles) and its degradation products was evaluated. BSA protein was added during the casting process (at 1.1% w/v BSA and 3% w/v PSA polymer) and in the growth medium at 1 mg/mL BSA was evaluated at 1:2 ratio of BSA to PSA (i.e., 1.1% w/v BSA and 3% w/v PSA). Experimental groups without BSA but with 3% w/v PSA or no PSA polymer served as controls.

2.4. Mechanical properties

The equilibrium Young's modulus (E_Y) and dynamic modulus (G*, at 1 Hz) were measured under unconfined compression (n = 3–7 per group) on a custom-built mechanical tester. A 10% strain was applied at a rate of 0.05 %/s and held for 30 min. The equilibrium stress was used to calculate the E_Y. Following stress-relaxation, the dynamic modulus was measured by imposing 2% sinusoidal strain at frequency 1 Hz.

2.5. Biochemical properties

Following mechanical testing, the sample's wet weight was obtained and prepared for biochemical analyses. Constructs were lyophilized for 48 h to obtain the dry weight and digested overnight using Proteinase K at 56° C. The agarose was separated by centrifugation as undigested mass and the supernatant was collected for biochemical analysis.

Glycosaminoglycan (GAG) content was assessed using 1,9-dimethylmethylene blue (DMMB) dye-binding assay for measuring sulfated-GAGs. The GAG content was quantified using chondroitin-6-sulfate standards and reported as a ratio of constructs' dry weight. The amount of DNA present in the samples was measured using PicoGreen assay.

2.6. Statistical Analysis

All reported data are presented as averages with standard deviations. A one-way ANOVA was performed on mechanical and biochemical properties. Once significance was found, a post hoc *t*-test was performed to compare experimental groups with the control.

3. Results and discussion

3.1. Characterization and release kinetics of polysebacic anhydride microparticles

The PSA microparticles were spherical with sizes ranging from several hundreds of nanometers to few tens of microns (Fig. 1). A 50% efficiency in amitriptyline encapsulation was observed during the fabrication process. The release profile of amitriptyline from the PSA microspheres was linear during the first two weeks (Fig. 2). Within 2 weeks, approximately 60% of the encapsulated amitriptyline was released from the PSA microparticles, after which the concentration of the released drug was too low for detection (Fig. 2). The fast release (<two weeks) and linear profile show that PSA-based polymeric microparticles can be utilized as carriers to provide sustained uniform delivery of nutrients.

3.2. Dose dependent cellular viability in the presence of PSA

The dose-dependence biocompatibility of PSA microparticles with chondrocytes was determined using a 3D agarose hydrogels. Initial cell viability observed within 24 h of hydrogel fabrication demonstrated little to no cell death for the 0.2% (2 mg/mL) PSA microparticles group (Fig. 3A). However, adding the PSA microparticles at a concentration of 2% (20 mg/mL) and 10% (100 mg/mL) resulted in widespread cell death throughout the construct (Fig. 3B & C). As expected, the drop in cell viability subsequently affected tissue growth and mechanical properties of engineered cartilage (Fig. 4; $p<0.01$ versus control). Equilibrium and dynamic moduli of the 0.2% w/v group were not significantly different from the control ($p = 0.43$). Taken together, these results indicate that the non-cytotoxicity of PSA polymeric microparticles is limited to concentrations below 0.2 % w/v (2 mg/mL).

In contrast to the earlier studies on biocompatibility of polyanhydrides [13,29–35], our results indicate cytotoxicity at higher concentrations of PSA microparticles. This agrees with recent work reported by Adler and coworkers [44], who found cytotoxic effects of polyanhydride copolymer (P(CPP-SA)) at concentrations at or above 2.8 mg/mL However, the mechanism of sebacic acid polymer cytotoxicity at higher concentrations is not well understood. Moreover, no methods have been proposed to mitigate cytotoxicity of PSA polymer.

3.3. Mechanisms of the cytotoxicity of PSA microparticles

Several factors contribute to inflammatory, cytotoxic, or genotoxic effect on cells, including changes in local pH, osmolality, disruption of cytoplasmic membrane, uptake of

biomaterials beyond biocompatible limits, Ca^{2+} levels, lipotoxicity, and induction of reactive oxygen or nitrogen species [46–48].

It is already known that a change in local pH from the physiological value of 7.2 significantly decreases matrix synthesis from chondrocytes [49]. In particular, the pH of our growth medium dropped from 7.5 to 6.3 when PSA-microparticles were suspended at 30 mg/mL for 5 h. However, increasing the buffer capacity of the culture medium (5 mM HEPES buffer) demonstrated that a drop in local pH was not the primary cause for cell death. HEPES buffer stabilized pH of the culture medium at 7.2, but did not did not improve cell viability in the presence of PSA microparticles (Supplementary information, Fig. S3).

The cytotoxicity of PSA may be due to cellular uptake of degradation products beyond biocompatible limits. The main degradation product of PSA is polysebacic acid, which can be considered a fatty acid-like molecule due to the presence of carboxylic acid groups and long hydrophobic chains. It is well known that excessive intracellular concentrations of alkyl fatty acids can cause abnormal levels of reactive oxygen species ROS and even result in cellular death [48,50], which is called lipotoxicity. If the chondrocytes ingest the PSA-microparticles or its degradation products, then the alkyl fatty acid-like concentrations inside cells can be expected to increase, causing lipotoxicity. The natural pathway by which cells cope with lipotoxicity is through the intracellular regulation of lipids by serum albumin proteins [51,52]. That is, by maintaining a ratio of serum albumin/lipids of 0.7–2, cells are able to combat the adverse effects caused by increased concentrations of fatty acids [52].

Thus, we hypothesized that if cytotoxicity was caused by lipotoxicity, the addition of bovine serum albumin (BSA) can mitigate cytotoxicity of PSA degradation products. To test this, BSA was added to the agarose hydrogel during casting and the culture medium was supplemented with 1 mg/mL of BSA throughout the culture period. BSA significantly decreased cytotoxicity from PSA microparticles at a concentration of 3% w/v (Fig. 5). At day 30, equilibrium compressive Young's and dynamic moduli of BSA-protected constructs were comparable to the PSA-free control (Fig. 5A, and B). The biochemical composition, GAG and DNA content (Fig. 5C, and 5D day 60), were also similar for these two groups.

These findings demonstrate that BSA can mitigate cytotoxic effects of PSA polymer, which presents a potential to use PSA microparticles as a delivery vehicle in engineered cartilage constructs. This finding of cytotoxic effects of hydrophobic anhydride polymers is important, since it has been earlier suggested that these polymers, when hydrolyzed to carboxylic acids, can act as nutrients for cells [53,54].

4. Conclusions

Polysebacic anhydride (PSA) polymer and PSA-based microparticles were evaluated for biocompatibility with chondrocytes and were tested for short-term (2 weeks) and sustained release of encapsulated nutrients within an agarose hydrogel for articular cartilage tissue engineering. PSA microparticles were biocompatible with chondrocytes at concentrations below 2 mg/mL and were found to be cytotoxic at a loading concentration of 20 mg/mL The mechanism of cytotoxicity is suggested to be due to the intracellular accumulation of PSA or its fatty acid-like hydrolysis products and the resulting lipotoxicity. Bovine serum albumin

(BSA) was found to offer the best protection from this adverse effect. Incorporating BSA into the hydrogel and culture system increased the usable PSA-microparticles towards 3% w/v (30 mg/mL) within the agarose hydrogel. In this study, we only evaluated one concentration of BSA with PSA polymer. Future work will evaluate whether BSA protection is maintained for higher doses of PSA polymer (e.g., 50–200 mg/mL). In conclusion, the findings reported here show the importance of determining dose dependent effects of PSA on biocompatibility and the potential use of PSA microparticles as a delivery vehicle for cartilage tissue engineering applications.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.colsurfb.2015.08.040.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Vunjak-Novakovic G, Altman G, Horan R, Kaplan DL. Tissue engineering of ligaments. Annu Rev Biomed Eng. 2004; 6:131–156. [PubMed: 15255765]
2. Hettrich CM, Crawford D, Rodeo SA. Cartilage repair third-generation cell-based technologies-basic science, surgical techniques, clinical outcomes. Sports Med Arthrosc. 2008; 16:230–235. [PubMed: 19011555]
3. Hung CT, Mauck RL, Wang CCB, Lima EG, Ateshian GA. A paradigm for functional tissue engineering of articular cartilage via applied physiologic deformational loading. Ann Biomed Eng. 2004; 32:35–49. [PubMed: 14964720]
4. O'Connell GD, Fong JV, Dunleavy N, Joffe A, Ateshian GA, Hung CT. Trimethylamine N-oxide as a media supplement for cartilage tissue engineering. J Orthop Res. 2012; 30:1898–1905. [PubMed: 22707357]
5. Buschmann MD, Gluzband YA, Grodzinsky AJ, Kimura JH, Hunziker EB. Chondrocytes in agarose culture synthesize a mechanically functional extracellular-matrix. J Orthop Res. 1992; 10:745–758. [PubMed: 1403287]
6. Bian L, Angione SL, Ng KW, Lima EG, Williams DY, Mao DQ, Ateshian GA, Hung CT. Influence of decreasing nutrient path length on the development of engineered cartilage. Osteoarthr Cartil. 2009; 17:677–685. [PubMed: 19022685]
7. Vert M. Aliphatic polyesters: Great degradable polymers that cannot do everything. Biomacromolecules. 2005; 6:538–546. [PubMed: 15762610]
8. Nair LS, Laurencin CT. Biodegradable polymers as biomaterials. Prog Polym Sci. 2007; 32:762–798.
9. Ng KW, O'Conor CJ, Kugler LE, Cook JL, Ateshian GA, Hung CT. Transient Supplementation of Anabolic Growth Factors Rapidly Stimulates Matrix Synthesis in Engineered Cartilage. Ann Biomed Eng. 2011; 39:2491–2500. [PubMed: 21833681]

10. Mauck RL, Nicoll SB, Seyhan SL, Ateshian GA, Hung CT. Synergistic action of growth factors and dynamic loading for articular cartilage tissue engineering. Tissue Eng. 2003; 9:597–611. [PubMed: 13678439]
11. Braun AG, Buckner CA, Emerson DJ, Nichinson BB. Quantative correspondence between the in vivo and in vitro activity of teratogenic agents. Proc Natl Acad Sci USA-Biol Sci. 1982; 79:2056–2060.
12. Domb AJ, Amselem S, Shah J, Maniar M. Polyanhydrides - Synthesis and characterization. Adv Polym Sci. 1993; 107:93–141.
13. Leong KW, Damore P, Marletta M, Langer R. Bioerodible polyanhydrides as drug-carrier matrices.2. Biocompatibility and chemical reactivity. J Biomed Mater Res. 1986; 20:51–64. [PubMed: 3949823]
14. Göpferich A, Langer R. The influence of microstructure and monomer properties on the erosion mechanism of a class of polyanhydrides. J Polym Sci Part A. 1993; 31:2445–2458.
15. Kumar N, Langer RS, Domb AJ. Polyanhydrides: an overview. Adv Drug Delivery Rev. 2002; 54:889–910.
16. Lopac SK, Torres MP, Wilson-Welder JH, Wannemuehler MJ, Narasimhan B. Effect of polymer chemistry and fabrication method on protein release and stability from polyanhydride microspheres. J Biomed Mater Res Part B. 2009; 91B:938–947.
17. Torres MP, Wilson-Welder JH, Lopac SK, Phanse Y, Carrillo-Conde B, Ramer-Tait AE, Bellaire BH, Wannemuehler MJ, Narasimhan B. Polyanhydride microparticles enhance dendritic cell antigen presentation and activation. Acta Biomater. 2011; 7:2857–2864. [PubMed: 21439412]
18. Torres MP, Determan AS, Anderson GL, Mallapragada SK, Narasimhan B. Amphiphilic polyanhydrides for protein stabilization and release. Biomaterials. 2007; 28:108–116. [PubMed: 16965812]
19. Kipper MJ, Wilson JH, Wannemuehler MJ, Narasimhan B. Single dose vaccine based on biodegradable polyanhydride microspheres can modulate immune response mechanism. J Biomed Mater Res. 2006; 76A:798–810.
20. Dang W, Daviau T, Brem H. Morphological characterization of polyanhydride biodegradable implant gliadel during in vitro and in vivo erosion using scanning electron microscopy. Pharm Res. 1996; 13:683–691. [PubMed: 8860422]
21. Akbari H, D'Emanuele A, Attwood D. Effect of geometry on the erosion characteristics of polyanhydride matrices. Int J Pharm. 1998; 160:83–89.
22. Domb AJ, Nudelman R. In-vivo and in-vitro elimination of aliphatic polyanhydrides. Biomaterials. 1995; 16:319–323. [PubMed: 7772672]
23. Leong KW, Brott BC, Langer R. Bioerodible polyanhydrides as drug-carrier matrices. 1. Characterization, degradation, and release characteristics. J Biomed Mater Res. 1985; 19:941–955. [PubMed: 3880353]
24. Mathiowitz E, Kline D, Langer R. Morphology of polyanhydride microsphere delivery systems. Scanning Microsc. 1990; 4:329–340. [PubMed: 2205908]
25. Park ES, Maniar M, Shah J. Effects of model compounds with varying physicochemical properties on erosion of polyanhydride devices. J Control Release. 1996; 40:111–121.
26. Shieh L, Tamada J, Chen I, Pang J, Domb A, Langer R. Erosion of a new family of biodegradable polyanhydrides. J Biomed Mater Res. 1994; 28:1465–1475. [PubMed: 7876286]
27. Tamada JA, Langer R. Erosion kinetics of hydrolytically degradable polymers. Proc Natl Acad Sci USA. 1993; 90:552–556. [PubMed: 8421690]
28. Domb AJ, Langer R. Solid-state and solution stability of poly(anhydrides) and poly(esters). Macromolecules. 1989; 22:2117–2122.
29. Laurencin C, Domb A, Morris C, Brown V, Chasin M, McConnell R, Lange N, Langer R. Poly(anhydride) administration in high-does in vivo - studies of biocompatibility and toxicology. J Biomed Mater Res. 1990; 24:1463–1481. [PubMed: 2279981]
30. Attawia MA, Herbert KM, Uhrich KE, Langer R, Laurencin CT. Proliferation, morphology, and protein expression by osteoblasts cultured on poly(anhydride-co-imides). J Biomed Mater Res. 1999; 48:322–327. [PubMed: 10398037]

31. Attawia MA, Uhrich KE, Botchwey E, Fan M, Langer R, Laurencin CT. Cytotoxicity testing of poly(anhydride-co-imides) for orthopedic applications. J Biomed Mater Res. 1995; 29:1233–1240. [PubMed: 8557725]
32. Domb AJ. Synthesis and characterization of biodegradable aromatic anhydride coplymers. Macromolecules. 1992; 25:12–17.
33. Domb AJ, Maniar M. Absorbable biopolymers derived from dimer fatty acids. J Poly Sci Part A-Polym Chem. 1993; 31:1275–1285.
34. Ibim SEM, Uhrich KE, Attawia M, Shastri VR, El-Amin SF, Bronson R, Langer R, Laurencin CT. Preliminary in vivo report on the osteocompatibility of poly(anhydride-co-imides) evaluated in a tibial model. J Biomed Mater Res. 1998; 43:374–379. [PubMed: 9855196]
35. Ibim SM, Uhrich KE, Bronson R, El-Amin SF, Langer RS, Laurencin CT. Poly(anhydride-co-imides): in vivo biocompatibility in a rat model. Biomaterials. 1998; 19:941–951. [PubMed: 9690836]
36. Braun AG, Buckner CA, Emerson DJ, Nichinson BB. Quantitative correspondence between the in vivo and in vitro acitivity of teratogenic agents. Proc Natl Acad Sci USA-Biol Sci. 1982; 79:2056–2060.
37. Domb AJ, Rock M, Perkin C, Yipchuck G, Broxup B, Villemure JG. Excretion of a radiolabeled anticancer biodegradable polymeric implant form the rabbit brain. Biomaterials. 1995; 16:1069–1072. [PubMed: 8519927]
38. Domb AJ, Rock M, Schwartz J, Perkin C, Yipchuk G, Broxup B, Villemure JG. Metabolic disposition and elimination studies of a radiolabeled biodegradable polymeric implant in the rat-brain. Biomaterials. 1994; 15:681–688. [PubMed: 7948590]
39. Ross R. Smooth muscle cell. 2. Growth of smooth muscle in culture and formation of elastic fibers. J Cell Biol. 1971; 50:172. [PubMed: 4327464]
40. Adler AF, Petersen LK, Wilson JH, Torres MP, Thorstenson JB, Gardner SW, Mallapragada SK, Wannemuehler MJ, Narasimhan B. High Throughput Cell-Based Screening of Biodegradable Polyanhydride Libraries. Comb Chem High Throughput Screen. 2009; 12:634–645. [PubMed: 19531023]
41. Determan AS, Trewyn BG, Lin VSY, Nilsen-Hamilton M, Narasimhan B. Encapsulation, stabilization, and release of BSA-FITC from polyanhydride microspheres, J. Control. Release. 2002; 100:97–109.
42. Lee YS, Johnson PJ, Robbins PT, Bridson RH. Production of nanoparticles-in-microparticles by a double emulsion method: A comprehensive study. Eur J Pharm Biopharm. 2013; 83:168–173. [PubMed: 23153669]
43. McCall RL, Sirianni RW. PLGA nanoparticles formed by single- or double-emulsion with vitamin E-TPGS. J Visualized Exp. 2013:51015.
44. Deo N, Somasundaran T, Somasundaran P. Solution properties of amitriptyline and its partitioning into lipid bilayers. Colloids Surf B: Biointerfaces. 2004; 34:155–159. [PubMed: 15261068]
45. Somasundaran P, Liu F, Chakraborty S, Gryte Carl C, Deo N, Somasundaran T. Novel Nanogels for Drug Binding and Delivery, Polymeric Drug Delivery II. Am Chem Soc. 2006:69–87.
46. Urban JPG, Hall AC, Gehl KA. Regulation of matrix synthesis rates by the ionic and osmotic environment of articular chondrocytes. J Cell Physiol. 1993; 154:262–270. [PubMed: 8425907]
47. Wu MH, Urban JPG, Cui ZF, Cui Z, Xu X. Effect of extracellular pH on matrix synthesis by chondrocytes in 3D agarose gel. Biotechnol Prog. 2007; 23:430–434. [PubMed: 17286385]
48. Almaguel FG, Liu JW, Pacheco FJ, Casiano CA, De Leon M. Activation and Reversal of Lipotoxicity in PC12 and Rat Cortical Cells Following Exposure to Palmitic Acid. J Neurosci Res. 2009; 87:1207–1218. [PubMed: 18951473]
49. Ohshima H, Urban JPG. The effect of lactate and pH on proteoglycan and protein-synthesis rates in the intervertebral-disk. Spine. 1992; 17:1079–1082. [PubMed: 1411761]
50. Prasad A, Bloom MS, Carpenter DO. Role of Calcium and ROS in Cell Death Induced by Polyunsaturated Fatty Acids in Murine Thymocytes. J Cell Physiol. 2010; 225:829–836. [PubMed: 20589836]
51. Choi JK, Ho J, Curry S, Qin DH, Bittman R, Hamilton JA. Interactions of very long-chain saturated fatty acids with serum albumin. J Lipid Res. 2002; 43:1000–1010. [PubMed: 12091483]

52. Spector AA. Fatty-acid binding to plasma albumin. J Lipid Res. 1975; 16:165–179. [PubMed: 236351]

53. Katti DS, Lakshmi S, Langer R, Laurencin CT. Toxicity, biodegradation and elimination of polyanhydrides. Adv Drug Deliv Rev. 2002; 54:933–961. [PubMed: 12384316]

54. Domb AJ, Rock M, Schwartz J, Perkin C, Yipchuk G, Broxup B, Villemure JG. Metabolic disposition and elimination studies of a radiolabeled biodegradable polymeric implant in the rat brain. Biomaterials. 1994; 15:681–688. [PubMed: 7948590]

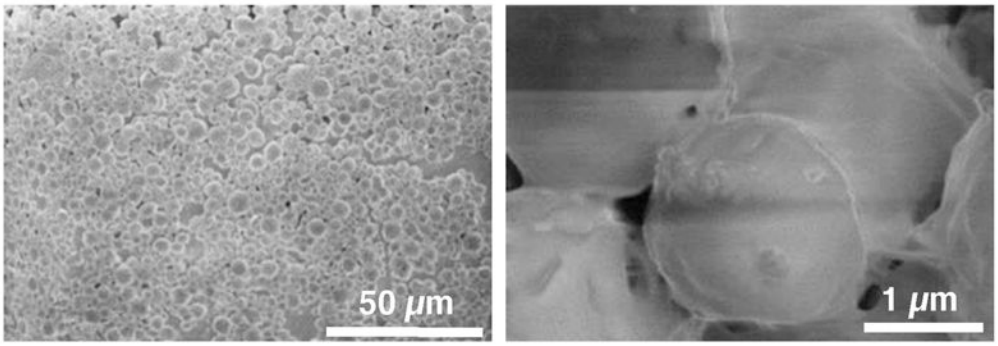

Fig. 1.

A. Representative scanning electron microscopy (SEM) images of PSA-microparticles. B. High resolution image of a single PSA-microparticle from the same batch.

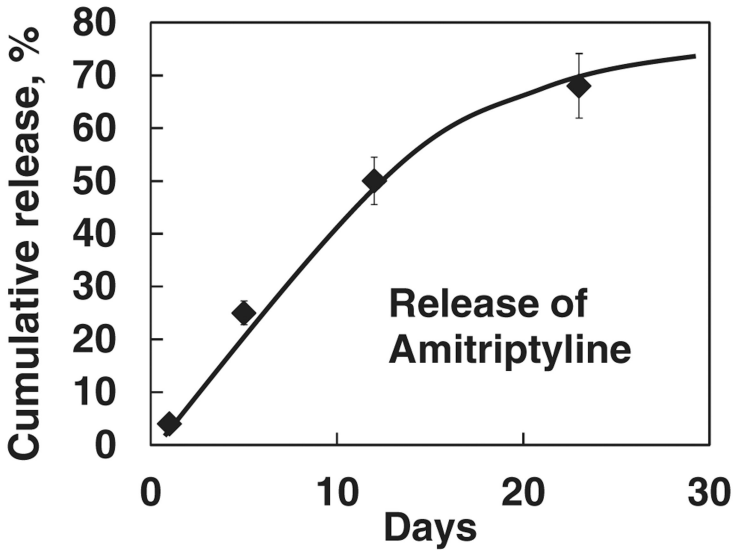

Fig. 2.
Amitriptyline release profile from PSA– microparticles (maximum standard deviation, SD = 9%, for $n = 4$).

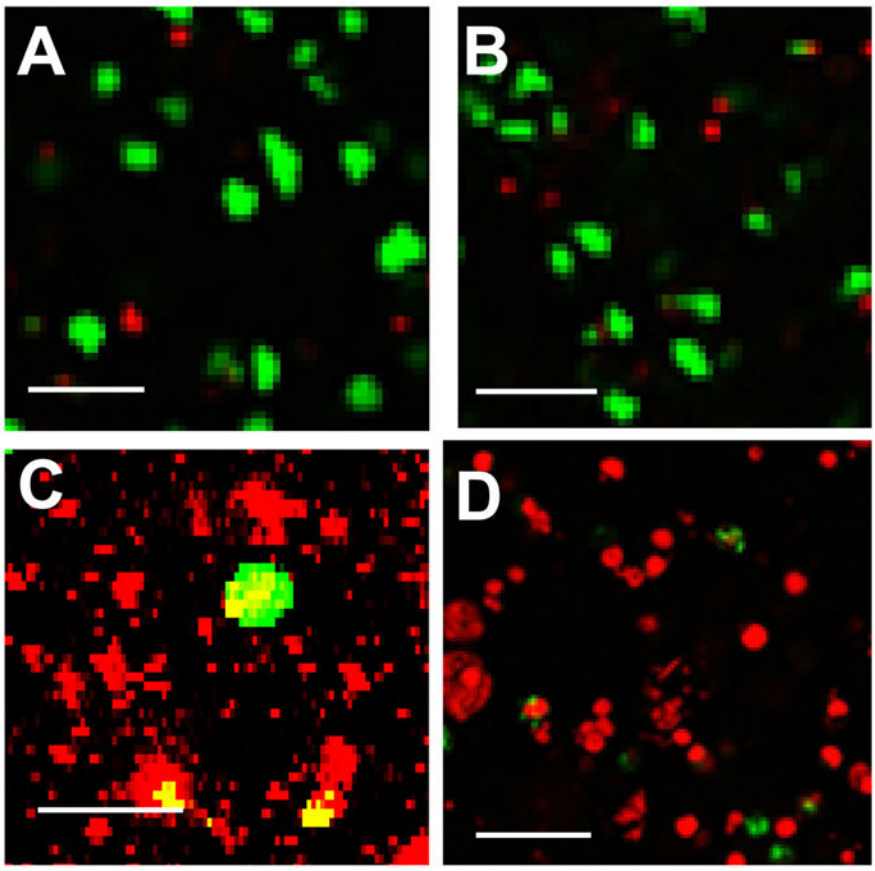

Fig. 3.

Cell viability of chondrocytes encapsulated in agarose hydrogel with PSA polymer at concentrations of (A) 0% w/v (control group), (B) 0.2% w/v (2 mg/mL) (C) 2% w/v (20 mg/mL) and (D) 10% w/v (100 mg/mL). Green color indicates living cells and red color indicates dead cells. Bar represents 50 μm.

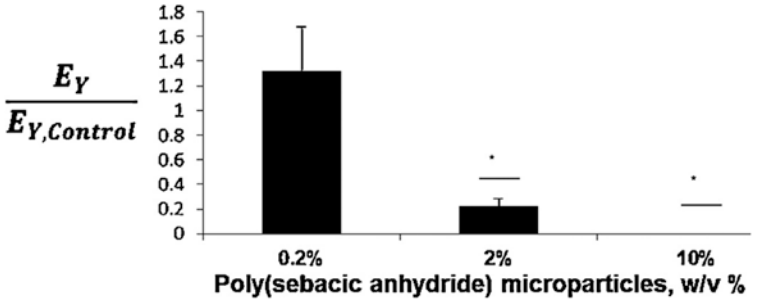

Fig. 4.

Compressive Young's modulus of engineered cartilage containing PSA-microparticles encapsulated with L-ascorbic acid 2-phosphate sesquimagnesium salt hydrate. Separate tissue engineering studies were run to evaluate each concentration group; therefore, the values were normalized to the respective control group (i.e., constructs without PSA; represented by the solid black line). The E_Y of 0.2, 2, and 10w/v% are reported for days 46, 52, and 14, respectively. The 10w/v% experimental group was discontinued after 14 days, due to lack of metabolic activity. SD = 2.5–10%, $n = 3$ per group. *Represents significant differences from the control group ($p < 0.01$).

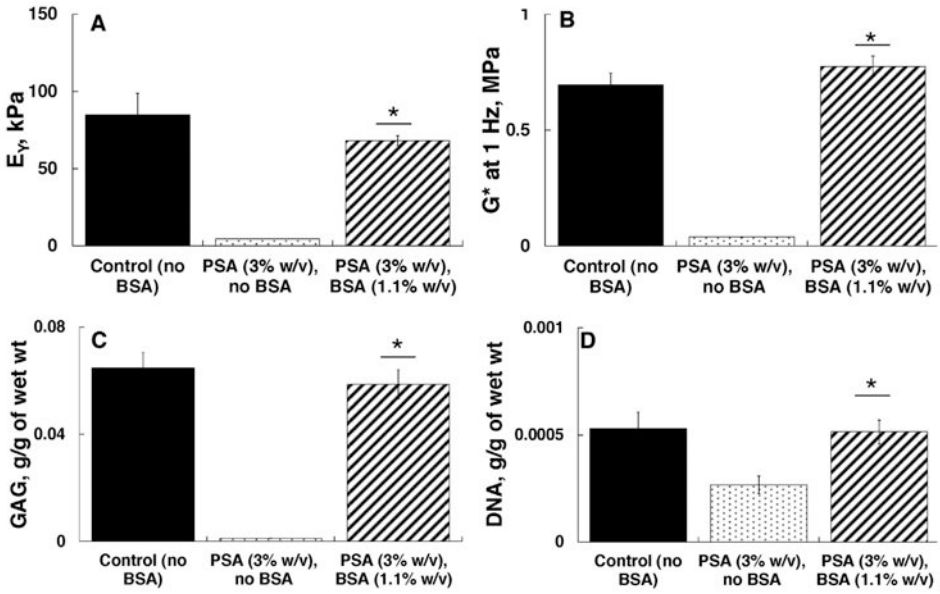

Fig. 5.

Compressive mechanical and biochemical properties of engineered cartilage constructs with BSA protection from PSA degradation byproducts. (A.) Equilibrium Young's modulus (E_Y), (B.) Dynamic modulus (G*), (C) GAG content, and (D) DNA content. Groups include BSA- and PSA-free control (solid), PSA-polymer without BSA (dots), and PSA-polymer with BSA (slant). The amount of GAG and DNA are reported in grams per gram of wet construct. SD = 2–16%, $n = 4$ per group.*Represents: No significant differences were observed between the control and BSA protected constructs ($p > 0.13$).