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On-demand drug delivery from self-assembled nanofibrous gels: A new approach for treatment of proteolytic disease

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Abstract: Local delivery of drugs offers the potential for high local drug concentration while minimizing systemic toxicity, which is often observed with oral dosing. However, local depots are typically administered less frequently and include an initial burst followed by a continuous release. To maximize efficiency of therapy, it is critical to ensure that drug is only released when needed. One of the hallmarks of rheumatoid arthritis, for example, is its variable disease activity consisting of exacerbations of inflammation punctuated by periods of remission. This presents significant challenges for matching localized drug delivery with disease activity. An optimal system would be nontoxic and only release drugs during the period of exacerbation, self-titrating in response to the level of inflammation. We report the development of an injectable self-assembled nanofibrous hydrogel, from a generally recognized as safe material, which is capable of encapsulation and release of agents in response to specific enzymes that are significantly upregulated in a diseased state including matrix metalloproteinases (MMP-2 and MMP-9) and esterases. We show that these self-assembled nanofibrous gels can withstand shear forces that may be experienced in dynamic environments such as joints, can remain stable following injection into healthy joints of mice, and can disassemble *in vitro* to release encapsulated agents in response to synovial fluid from arthritic patients. This novel approach represents a next-generation therapeutic strategy for localized treatment of proteolytic diseases. © 2011 Wiley Periodicals, Inc. J Biomed Mater Res Part A: 97A: 103–110, 2011.

Key Words: hydrogel, self-assembly, controlled release, arthritis, drug delivery, inflammation

INTRODUCTION

Delivering drugs to patients in a safe, effective, and compliant manner is a major challenge for the treatment of many types of disease.[1] The ability of drugs to reach target tissues from the point of oral administration is limited by multiple barriers including enzymatic and acidic degradation in the stomach, absorption across the intestinal epithelium, hepatic clearance, and nonspecific uptake. Effective oral dosing to achieve high concentrations of drugs within specific tissues while minimizing systemic toxicity remains a significant challenge. Conventional

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polymeric drug delivery systems such as implants, injectable microspheres, and patches are used by tens of millions of people annually, yet often produce a sharp initial increase in concentration to a peak above the therapeutic range, followed by a fast decrease in concentration to a level below the therapeutic range.[1] Additionally, noncompliance with oral medication is a leading cause of hospitalizations. The Holy Grail of drug delivery is an autonomous system that titrates the amount of drug released in response to a biological stimulus, ensuring that the drug is released only when needed at a therapeutically relevant concentration. Such a system must rapidly release drug in response to fluctuations due to the severity of disease (this is often reflected by the local inflammatory state), patient-to-patient variability, and environmental factors.

There exist broad implications for achieving an on-demand drug delivery approach for the treatment of tissue defects and multiple diseases. One approach toward this goal is the design of compounds tailored to release drugs in response to the local expression of enzymes that correlate with the level of inflammation. Inflammatory conditions that are characterized by the generation of enzymes that destroy extracellular connective tissue—such as rheumatoid arthritis (RA) and wound healing—comprise a particularly attractive first application. By targeting other disease-associated enzyme pathways, this platform would have broad applicability for cancer, ocular disease, oral disease, gastrointestinal disease, and cardiovascular disease. One of the hallmarks of RA, for example, is its variable disease activity consisting of exacerbations (flare-ups) of the chronic inflammatory joint process punctuated by periods of remission. As most disease-modifying drugs in RA demonstrate slow onset (often weeks), treatment options during flare-ups are limited and often use corticosteroids—agents with a plethora of toxic side effects.[2,3] With a prevalence of 1%, RA is the most common form of human inflammatory arthritis. In the United States alone, it is estimated that 2.5 million people suffer from the disease, with a monetary cost measured in billions.[4] Although there have been significant advances in basic science and therapeutics, current treatments remain only partially effective and plagued by dose- or duration-limiting toxicity. This is exemplified by the removal of drugs such as Vioxx and Bextra from the market and the Food and Drug Administration (FDA)'s recent rejection of Arcoxia for market approval. Intraarticular injections may help reduce systemic toxicity given the reduced drug amount required to exert local activity within the joint space. Nevertheless, their therapeutic impact is often short lived because of rapid clearance from the joint ($t_{1/2} \sim 0.1$–6 h),[5] and existing drug delivery depots have poor physical stability within the joint.[5]

Thus, the use of a long-acting intraarticular drug delivery method that titrates release to match disease activity and accommodates a large quantity of drug would represent an attractive paradigm shift. Importantly, published literature has demonstrated that specific enzymes are significantly upregulated within arthritic joints such as matrix metalloproteinases 2 and 9 (MMP-2 and MMP-9),[6] and their expression and concentration correlate with the degree of synovial inflammation.[7] Current approaches for releasing drugs in response to specific stimuli (e.g., MMP-sensitive crosslinked hydrogels) show promise,[8–11] but typically only accommodate low concentrations of drugs and crosslinked gels present slow diffusion times.

We have focused our attention on the development of enzyme-responsive self-assembled nano/microfibrous hydrogels that can easily be injected into the articular space, yet are much larger than free drug, which should increase residence time by preventing rapid clearance by the lymphatic system. The inherent nanometer-scale features of this self-assembled noncrosslinked hydrogel maximize the interaction with specific enzymes for rapid disassembly and drug release. In this proof-of-concept work, we have identified an amphiphilic low-molecular-weight agent from the United States FDA's generally recognized as safe (GRAS) list of agents that can serve as a highly efficient hydrogelator to entrap and release model agents in response to enzymes that are present during inflammatory conditions. In particular, we have shown that gels made from ascorbyl palmitate (Asc-Pal) can encapsulate model agents, withstand shear forces that may be experienced in dynamic environments such as joints, remain stable following injection into healthy joints of mice, and can disassemble *in vitro* to release encapsulated agents in response to synovial fluid from arthritic patients.

MATERIALS AND METHODS

General information

Asc-Pal and matrix metalloproteinases were purchased from Sigma Aldrich (St. Louis, MO). The Novozyme 435 (lipase B from *Candida antarctica*) and Lipolase 100L were obtained from Novozymes through Brenntag North America. 1,1′-Dioctadecyl-3,3,3′,3′-tetramethylindodicarbocyanine, 4-chlorobenzenesulfonate salt (DiD) dye was purchased from Invitrogen. All other chemicals and reagents were purchased from Sigma Aldrich (St. Louis, MO) and were used without further modification or purification unless otherwise specified.

Preparation of gels

Given that Asc-Pal has not previously been shown to self-assemble into hydrogels, multiple solvent systems were attempted. Typically, solvents (0.2 mL) were added to a glass scintillation vial with the gelator (0.5–5 wt/vol %) and sealed with a screw cap. The vial was heated to ~60–80°C until the gelator was completely dissolved. The vial was placed on a stable surface and allowed to cool to room temperature. Typically after 15–45 min, the solution was transitioned into a viscous gel. Gelation was considered to have occurred when no gravitational flow was observed upon inversion of the glass vial, and resulted hydrogels are injectable.

Scanning electron microscopy

For electron microscopic analysis of gel morphology, solid gels derived with unhindered shrinkage (xerogels) were prepared by lyophilizing the gels (which were prepared as described above). Small amounts of xerogel were placed on carbon tape attached to aluminum grids and coated with a thin layer of gold using a sputtering machine. Those aluminum grids were directly imaged under scanning electron microscopy (SEM).

UV–vis spectroscopy

UV–visible spectra of the amphiphile gelators and DiD dye were recorded using a CARY100BIO spectrophotometer. In all experiments, solutions were observed in a quartz cuvette with a 1-cm path length.

X-ray diffraction

X-ray diffraction (XRD) measurements were conducted using a Bruker AXS D-8 Discover with a GADDS diffractometer using graded *d*-space elliptical side-by-side multilayer optics, monochromated Cu-Kα radiation (40 kV, 40 mA), and imaging plate.

Ab initio calculations

All *ab initio* Hartree–Fock calculations reported in this work were performed using the Gaussian 03 suite program.[12] The geometry of Asc-Pal was located and optimized at the level of restricted Hartree–Fock using the 6-31G* basis set. The structure of Asc-Pal was completely optimized without any symmetry restrictions. Vibrational frequency calculations were performed to confirm that they converge to true minima by diagonalization of their Hessian (force constant) matrixes at the same level to ensure that all frequencies were genuine.

Release kinetics

DiD-encapsulating Asc-Pal self-assembled gel fibers (200 μL) were suspended in PBS (800 μL), and either MMP-2, MMP-9, or lipase enzyme (100 ng/mL) was added followed by incubation at 37°C. At each time point, an aliquot (10 μL) from the supernatant above the fibers was dissolved in DMSO (90 μL), and the released DiD was quantified by UV–vis spectroscopy at the characteristic wavelength of 655 nm. After withdrawing each aliquot, the incubation medium was replenished with PBS (10 μL). To examine the potential for on-demand drug release, enzyme-containing media were removed after a 4-day incubation and replenished with PBS. After 7 days, fresh enzyme was added.

Human synovial fluid analysis

Human knee synovial fluids were obtained as discarded materials from patients with RA undergoing diagnostic or therapeutic arthrocentesis. Arthritis diagnosis was ascertained by an American Board of Internal Medicine certified Rheumatologist and/or by review of laboratory, radiologic, and clinic notes and by applying ACR classification criteria.[13] All studies received Institutional Review Board approval.

Stability of self-assembled gel fibers in mice

Six 9-week-old Balb/C mice housed in a specific pathogen-free animal facility at the Dana-Farber Cancer Institute were used for these studies. All procedures were approved by the Institutional Animal Care and Use Committee of the Dana-Farber Cancer Institute (Boston, MA). Self-assembled fibers were redispersed in PBS solution and injected using a 27-gauge needle into the ankles of Balb/C mice. Animals were sacrificed after 8 weeks and thick 20- to 30-μm cryosections were obtained. Care was taken to avoid the use of alcohols or organic solvents that could dissolve the gels and compromise visualization of intact gel fibers. Thus, unstained sections were examined for fluorescence from DiD dye. For the evaluation of fiber disassembly by murine arthritic joint proteins, we prepared lysates in the absence or presence of protease inhibitor cocktail (Sigma) as described[14] using arthritic ankles from 7-week-old K/BxN mice.[15]

RESULTS AND DISCUSSION

One of the greatest challenges in the field of drug delivery is that many drugs have been deemed unsuitable for oral dosing given their systemic toxicity profile. Often this is due to the high concentrations required to produce a therapeutic effect at a localized site. Localized delivery of drugs offers significant advantages for the treatment of many diseases; however, there have been very few approved implantable or injectable local drug delivery systems. Drug delivery depots that can effectively achieve high concentrations of drugs within specific environments while limiting systemic toxicity would be useful for multiple applications. Self-assembled gels from amphiphilic gelators represent an attractive platform for localized controlled drug delivery given their inherent nanofibrous morphology with a high surface area to volume ratio that permits ease of injection and helps to promote rapid drug release in an enzyme-responsive manner. Although there are many approaches to develop self-assembled gels through synthesis of novel gelators, we propose a conceptually novel approach through selecting amphiphiles from agents that have already been used in humans with an established safety profile. Specifically, we selected an agent from the FDA's list of GRAS agents that we believed was a candidate hydrogelator capable of forming gels that could be disassembled in response to enzymes that are typically upregulated in proteolytic diseases including MMPs and esterases. We selected Asc-Pal given that it exhibits both hydrophobic and hydrophilic domains (amphiphilic) and has potential for self-assembly given its ability to form noncovalent interactions including van der Waals forces and hydrogen bonding.

Design and gelation studies of Asc-Pal amphiphile

Asc-Pal, also known as 6-*O*-palmitoyl ascorbic acid (see Fig. 1), was selected as a GRAS-based amphiphilic gelator. Asc-Pal encompasses the structural features required for the self-assembly, for example, a polyhydroxyl sugar head group for the formation of a hydrogen-bonding network, and a polymethylene hydrocarbon chain for van der Waals interactions. These groups synergistically act to form strong intermolecular interactions that have the potential to induce gelation (formation of nano/microfibrous structures). In addition, Asc-Pal has an ester linkage that enables cleavage by esterases and MMPs that are present during an inflamed condition (Fig. 1). Use of self-assembled gels from amphiphilic gelators provides an opportunity to avoid the undesired burst release[16] that is often the characteristic limitation of drug delivery devices.[17] Asc-Pal showed excellent gelation ability as assessed in a wide range of solvents at

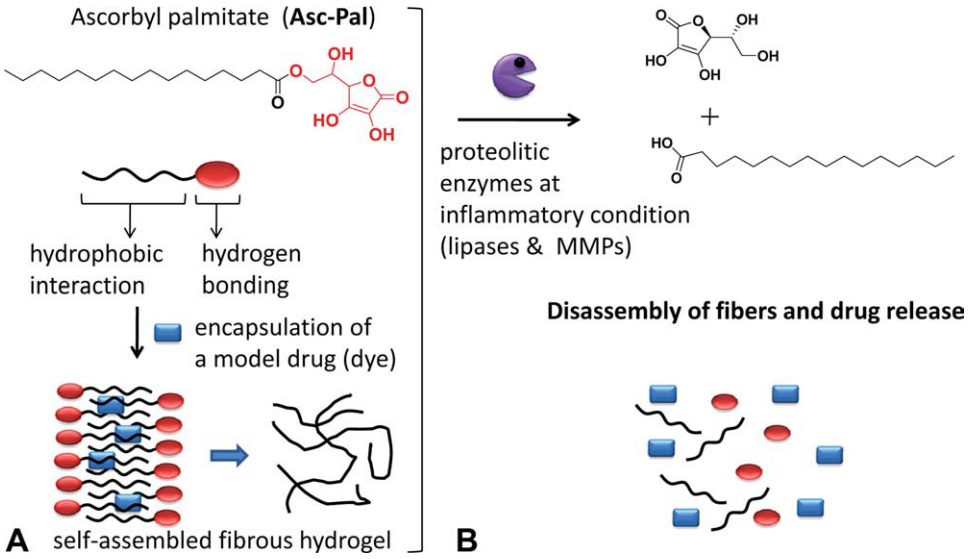

FIGURE 1. (A) Chemical structure of Asc-Pal amphiphile, and schematic representation of the encapsulation of dye during the self-assembly process. (B) Schematic of enzyme-triggered drug (dye) release through degradation of self-assembled fibers. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

low concentrations (2–5 wt/vol %) as shown in Table I. Additionally, a dye could be added during the assembly process as a model drug, enabling direct imaging of the fibers.

The morphologies of the self-assembled hydrogels were examined using SEM and fluorescence polarizable optical microscopy. Investigation of the hydrogels formed from Asc-Pal with SEM showed that hydrogels form fibrous structures with fiber thicknesses of 20–300 nm and fiber lengths of several microns [Fig. 2(A)]. The anisotropic nature of intermolecular interactions between amphiphile molecules is supported by the high aspect ratios of the gel fibers. Dye-encapsulating fibers were rinsed with excess PBS to remove unencapsulated dye, and subsequent fluorescence microscope images of the fibers [Fig. 2(B)] indicated that the dye was encapsulated within the fibers.

To propose a model for self-assembly of amphiphiles in aqueous solution, we calculated long distance spacings (*d*) from the XRD patterns of the hydrogel of Asc-Pal. We obtained optimized geometries and calculated the length of amphiphile using *ab initio* calculations and by combining these results with the data from XRD. Through this, we propose a model for the self-assembly of Asc-Pal. XRD experiments [Fig. 3(A)] yielded a long distance spacing of 4.39 nm for hydrogel fibers of Asc-Pal, which is higher than the molecular length (2.60 nm from optimized structure) of Asc-Pal and lower than double the extended molecular length. Thus, we postulated that a highly interdigitated bilayer-like structure is present at the molecular level [Fig. 3(B)].

Enzyme-responsive fiber disassembly and release of dye

In addition to rapid clearance of drugs from joints, which is a major limitation of intraarticular injections,[18] conventional drug delivery systems that aim to control drug release typically result in an initial burst[17] followed by continuous release, which is not ideal for many applications involving fluctuations in the disease state (i.e., drug continues to release even when it is not needed). To address these limitations, it is important to develop a drug delivery system that may reside within the joint and release drugs in response to the status of the disease. In this work, we demonstrate the encapsulation of a model dye in Asc-Pal hydrogel, which upon enzyme-mediated gel degradation releases the encapsulated dye at model physiological conditions in a controlled manner. Previously, we have demonstrated the enzyme-triggered controlled delivery of hydrophobic drugs using molecular hydrogels.[19] To investigate enzyme-responsive release from Asc-Pal-based self-assembled gel fibers, DiD (absorption λ_{max} of 655 nm) was selected as a model small molecule (MW = 1052) for encapsulation. Specific enzymes are significantly upregulated within arthritic joints,[6] and their expression and concentration correlate with the degree of synovial inflammation.[7] Thus, we have tested the ability of self-assembled gel fibers to release an encapsulated payload in response to the enzymes that are expressed

TABLE I. Gelation Ability of GRAS-Amphiphile Asc-Pal in Various Solvents

Solvent	Asc-Pal
Water	G
Benzene	G
Toluene	G
Carbon tetrachloride	G
Acetonitrile	G
Chloroform	S
Methanol	S
Dimethylformamide	P
Dimethylsulfoxide	P

Solutions were considered to gel only if upon inversion of the glass vial the solution did not flow in response to gravity.
G, gel; P, precipitate; S, soluble.

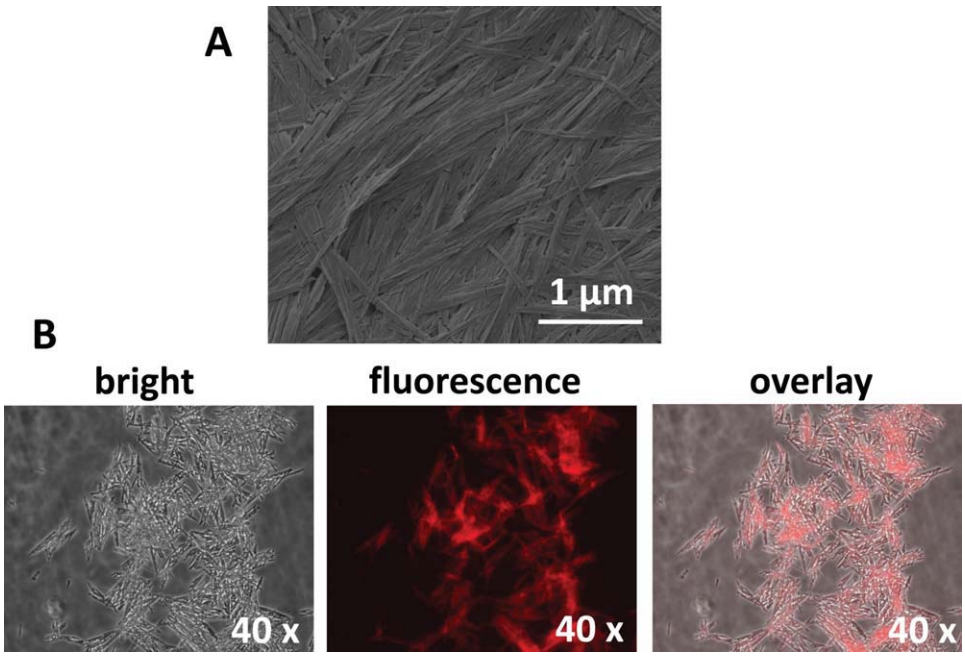

FIGURE 2. (A) Scanning electron micrograph and (B) brightfield and fluorescence optical images of dye (DiD)-encapsulating self-assembled fibers from Asc-Pal. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

within arthritic joints. DiD-encapsulating gel fibers were dispersed within PBS and incubated at 37°C with either lipase (esterase), or MMP-2, or MMP-9 enzyme (100 ng/mL). At regular intervals, aliquots of samples were collected, and release of the dye was quantified using absorption spectroscopy. Plotting cumulative release of the dye (%) versus time [Fig. 4(A)] revealed that lipase and MMPs trigger fiber disassembly to release the encapsulated dye, whereas gels in PBS controls remained stable and did not release significant amounts of dye. Additionally, through thin-layer chromatography, we identified the presence of ascorbic acid and palmitic acid (confirmed by comparing R_f values by cospotting with authentic samples of ascorbic and palmitic acids) only in gel solutions that contained enzymes. We have shown that gels in PBS remain stable for at least 3 months, indicating that the presence of enzymes is required for gel disassembly and the release of encapsulated agents. This result confirms the absence of loosely bound dye on the surface of the gel fibers. In the absence of enzymes, mechanical agitation of the fibers through rigorous vortexing did not induce the release of dye, indicating that agents incorporated within the fibers remain stably entrapped. Importantly, in the present system, we did not observe burst release (Fig. 4), which is consistent with self-assembled prodrug-based gels that we have previously

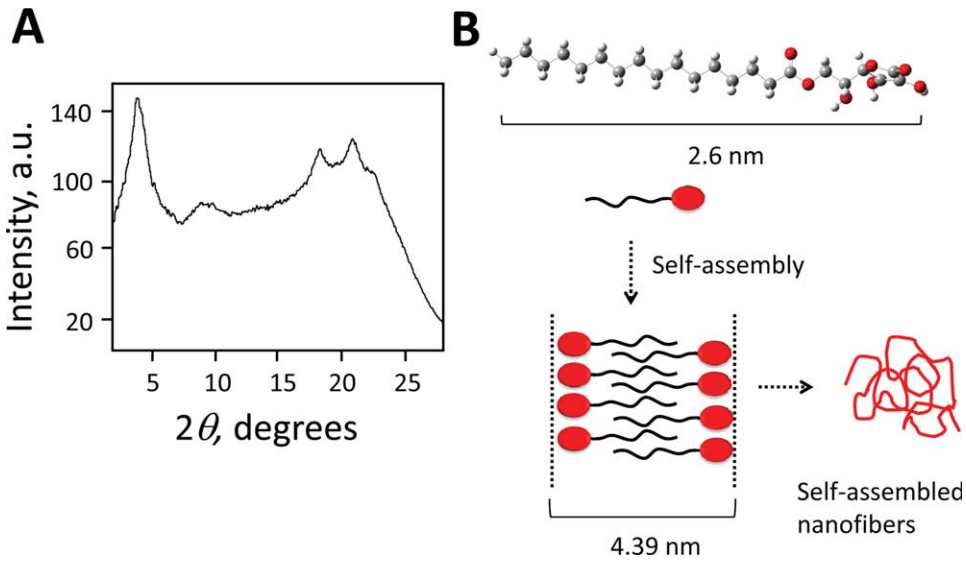

FIGURE 3. (A) X-ray diffraction plot of self-assembled hydrogels made from Asc-Pal. (B) Top: Optimized structure of Asc-Pal; bottom: postulated mode of self-assembly of Asc-Pal within an aqueous solution. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

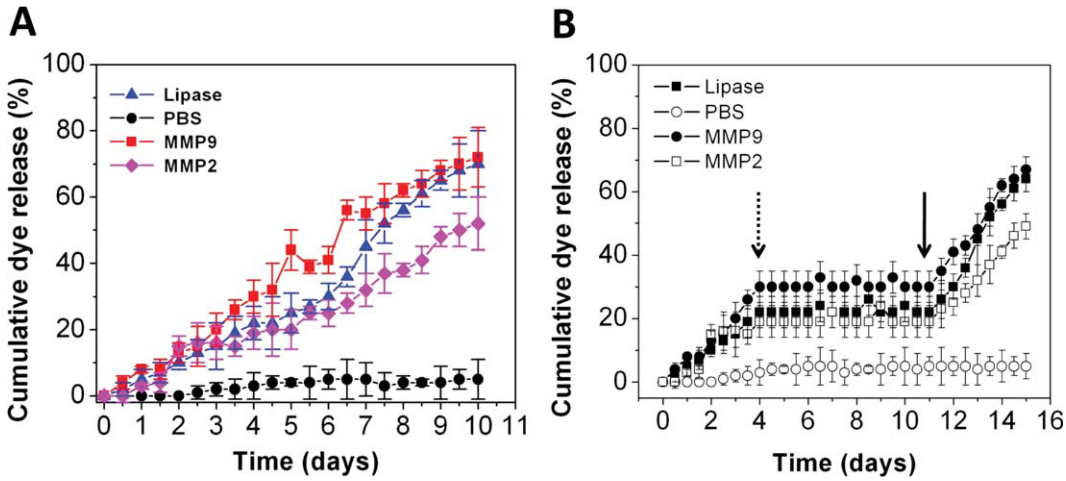

FIGURE 4. DiD release from Asc-Pal self-assembled gel fibers in response to lipase, MMP-2, and MMP-9 at 37°C *in vitro*. (A) Enzyme was added on day 0, and release kinetics were continuously monitored. (B) After 4 days of enzyme addition, media were changed to remove the enzyme (dotted arrow); on day 11, fresh enzyme was added (solid arrow), triggering the release of dye. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

synthesized.[19] To investigate the potential for on-demand disassembly, following a 4-day incubation with enzyme (MMP-2, MMP-9, or lipase) containing media that triggered disassembly of fibers, media were replaced with PBS, which halted the disassembly of fibers and the release of dye. After a subsequent 7-day incubation with PBS, enzymes were added to the suspended fibers, triggering disassembly and the release of the encapsulated dye [Fig. 4(B)]. These results clearly suggest that Asc-Pal self-assembled fibers respond to proteolytic enzymes that are present within arthritic joints and release encapsulated agents in an on-demand manner.

Arthritic synovial fluid induces fiber disassembly

To investigate whether arthritic synovial fluid could trigger fiber disassembly, leading to the release of the encapsulated dye, we obtained synovial fluid from arthritic human joints. DiD-encapsulating fibers were incubated in arthritic synovial fluid at 37°C, and the release of dye was quantified over a period of 15 days. Plotting cumulative release of the dye (%) versus time [Fig. 5(A)] revealed that synovial fluid triggers fiber disassembly leading to the release of the dye. To determine whether proteases that are present in arthritic joints were responsible for fiber disassembly, we prepared lysates from arthritic joints of mice in the presence and absence of protease inhibitors. Incubation of self-assembled gel fibers with these lysates was used to help reveal the role of arthritis-associated proteases. The presence of protease inhibitors significantly reduced fiber disassembly and dye release, thus demonstrating that the presence of enzymes was critical for promoting the release of agents from gels formed from Asc-Pal [Fig. 5(B)].

Fiber stability in joints under nonarthritic conditions

To investigate the stability of fibers in the absence of inflammation, fibers were injected into the joints of healthy mice using a small-bore (27 gauge) needle. Eight weeks postimplantation, the ankles of mice were sectioned and imaged with optical and fluorescence microscopy to observe

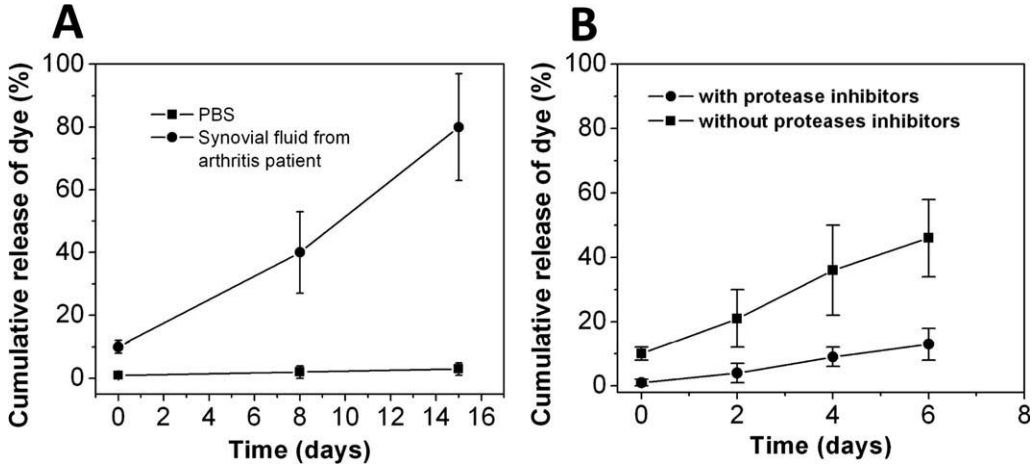

FIGURE 5. (A) Synovial fluid collected from arthritis patients mediated fiber disassembly and dye release over a 15-day period, whereas dye was not released from gels incubated with PBS. (B) Gel fibers were incubated with synovial lysates prepared from ankle tissue of arthritic mice with and without protease inhibitors. The presence of protease inhibitors significantly reduced the release of dye from the Asc-Pal self-assembled gel fibers.

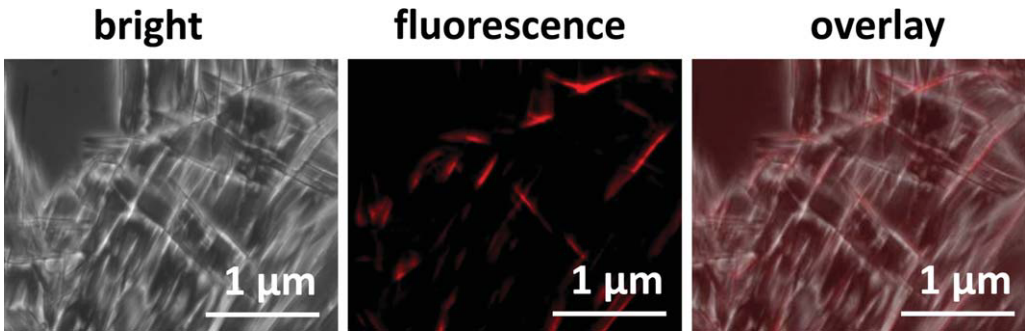

FIGURE 6. Fluorescence optical microscope images of harvested ankles of healthy mice 8 weeks following local injection. Fluorescence was not detected in joints that were injected with gel fibers in the absence of encapsulated dye (not shown). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

the presence of fibers. Images of tissue sections (Fig. 6) revealed that DiD-encapsulating fibers were present, suggesting the potential for long-term hydrolytic stability of the fibers *in vivo*.

Reversible self-assembly of fibers

Materials that are injected into the joint space experience cyclical mechanical forces during ambulation; thus, it is important that materials that are injected into the joint can withstand these forces and retain their characteristic material properties such as mechanical strength and morphology. To investigate the impact of relevant mechanical forces on the Asc-Pal fibers, we subjected gel nanofibers to cyclical shear forces and examined their resulting rheological properties using a rheometer equipped with a parallel-plate geometry [Fig. 7(A)]. The elastic/storage modulus G' was independent of frequency and was much higher than the viscous modulus G'' over the frequency range (0–12 rad/s) examined [Fig. 7(A)]. This type of response is typical of gels,[20,21] as it shows that the sample does not change its properties or "relax" over long time scales. The value of G' is a measure of the gel stiffness, and its value here ($>$1000 Pa) indicates a gel of slightly higher strength than collagen-platelet gels.[22] The mechanical properties and strength of these gels are comparable with earlier reported self-assembled peptide gels that are being examined as possible injectable joint lubricants for the treatment of osteoarthritis.[23] Frequency sweeps conducted before/after multiple cycles (1, 20, and 40) of a high shear stress were used to measure G' (storage modulus). Interestingly, no significant differences were observed after 40 cycles [Fig. 7(B)], indicating that the gel fibers retain their mechanical strength. These results indicate that self-assembled fibers made of Asc-Pal have the potential to retain their morphology and mechanical properties even under the dynamic forces that may be experienced during ambulation.[24,25]

CONCLUSION

Herein, we demonstrated that an amphiphilic GRAS agent, Asc-Pal, represents an efficient low-molecular-weight hydrogelator capable of encapsulation of model agents through self-assembly within an aqueous solution. The resultant gel should exhibit minimal toxicity given that it disassembles into readily metabolized ascorbic and palmitic acids. The molecular properties of the self-assembled gels were assessed through analysis with XRD and theoretical calculations. Upon self-assembly, Asc-Pal formed a nano/microfibrous hydrogel that could easily be injected through a

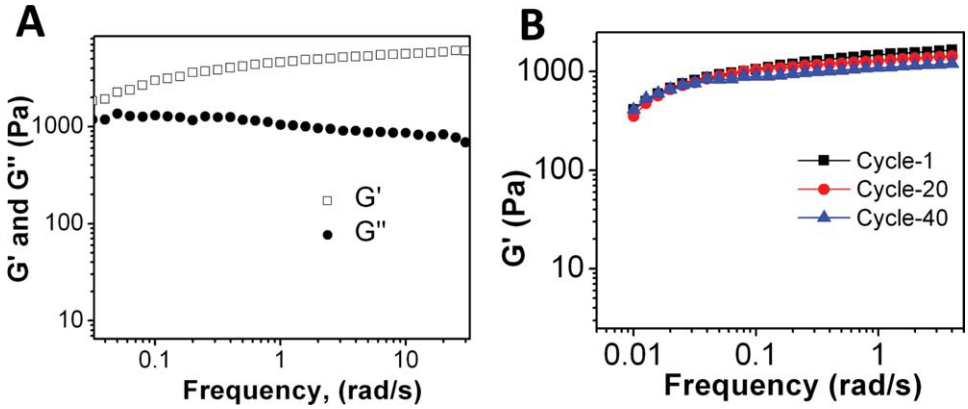

FIGURE 7. Dynamic rheology of Asc-Pal fibrous hydrogels assessed with a parallel-plate rheometer. (A) Storage modulus, G', and viscous modulus, G'', over a frequency range of 0–12 rad/s. (B) Frequency sweeps conducted before/after multiple cycles of a high shear stress to measure G'. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

small-bore needle (27 gauge), and dynamic rheology studies suggested that fibers retained their mechanical properties under multiple cycles of compression. The Asc-Pal gels remained stable within normal joints for at least 8 weeks, yet disassembled and released encapsulated agents in response to enzymes that are known to be overexpressed during flares of RA, and in the presence of synovial fluid from arthritic human joints. Further *in vivo* experiments using arthritic mice are currently underway. The use of self-assembled gels developed from low-molecular-weight hydrogelators to locally deliver drugs in an enzyme-responsive manner should have broad implications for the localized treatment of many proteolytic diseases. For example, drug release may be triggered for treatment of tumors as a result of the enzymatic action of tumor cell-associated proteases[26,27] such as plasmin, or drug may be programed to release in selected sites of the gastrointestinal tract under the influence of specific digestive enzymes.[28,29]

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REFERENCES

1. Langer R. Where a pill won't reach. Sci Am 2003;288:50–57.
2. Wolfe F, Caplan L, Michaud K. Treatment for rheumatoid arthritis and the risk of hospitalization for pneumonia: Associations with prednisone, disease-modifying antirheumatic drugs, and anti-tumor necrosis factor therapy. Arthritis Rheum 2006;54:628–634.
3. Coulson KA, Reed G, Gilliam BE, Kremer JM, Pepmueller PH. Factors influencing fracture risk, T score, and management of osteoporosis in patients with rheumatoid arthritis in the Consortium of Rheumatology Researchers of North America (CORRONA) registry. J Clin Rheumatol 2009;15:155–160.
4. Lipsky PE, Kavanaugh A. The impact of pharmaco-economic considerations on the utilization of novel anti-rheumatic therapies. Rheumatology (Oxford) 1999;38(Suppl 2):41–44.
5. Larsen C, Ostergaard J, Larsen SW, Jensen H, Jacobsen S, Lindegaard C, Andersen PH. Intra-articular depot formulation principles: Role in the management of postoperative pain and arthritic disorders. J Pharm Sci 2008;97:4622–4654.
6. Tchetverikov I, Ronday HK, Van El B, Kiers GH, Verzijl N, TeKoppele JM, Huizinga TW, DeGroot J, Hanemaaijer R. MMP profile in paired serum and synovial fluid samples of patients with rheumatoid arthritis. Ann Rheum Dis 2004;63:881–883.
7. Maeda S, Sawai T, Uzuki M, Takahashi Y, Omoto H, Seki M, Sakurai M. Determination of interstitial collagenase (MMP-1) in patients with rheumatoid arthritis. Ann Rheum Dis 1995;54: 970–975.
8. Liu XM, Yang YY, Leong KW. Thermally responsive polymeric micellar nanoparticles self-assembled from cholesteryl end-capped random poly(*N*-isopropylacrylamide-*co*-*N*,*N*-dimethylacrylamide): Synthesis, temperature-sensitivity, and morphologies. J Colloid Interface Sci 2003;266:295–303.
9. Ulijn RV. Enzyme-responsive materials: A new class of smart biomaterials. J Mater Chem 2006;16:2217–2225.
10. Seliktar D, Zisch AH, Lutolf MP, Wrana JL, Hubbell JA. MMP-2 sensitive, VEGF-bearing bioactive hydrogels for promotion of vascular healing. J Biomed Mater Res A 2004;68:704–716.
11. Bikram M, West JL. Thermo-responsive systems for controlled drug delivery. Expert Opin Drug Deliv 2008;5:1077–1091.
12. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, Cheeseman JR, Montgomery JA Jr , Vreven T, Kudin KN, Burant JC, Millam JM, Iyengar SS, Tomasi J, Barone V, Mennucci B, Cossi M, Scalmani G, Rega N, Petersson GA, Nakatsuji H, Hada M, Ehara M, Toyota K, Fukuda R, Hasegawa J, Ishida M, Nakajima T, Honda Y, Kitao O, Nakai H, Klene M, Li X, Knox JE, Hratchian HP, Cross JB, Adamo C, Jaramillo J, Gomperts R, Stratmann RE, Yazyev O, Austin AJ, Cammi R, Pomelli C, Ochterski JW, Ayala PY, Morokuma K, Voth GA, Salvador P, Dannenberg JJ, Zakrzewski VG, Dapprich S, Daniels AD, Strain MC, Farkas O, Malick DK, Rabuck AD, Raghavachari K, Foresman JB, Ortiz JV, Cui Q, Baboul AG, Clifford S, Cioslowski J, Stefanov BB, Liu G, Liashenko A, Piskorz P, Komaromi I, Martin RL, Fox DJ, Keith T, Al-Laham MA, Peng CY, Nanayakkara A, Challacombe M, Gill PMW, Johnson B, Chen W, Wong MW, Gonzalez C, Pople JA. Gaussian, Inc., Pittsburg, PA, 2003.
13. Arnett FC, Edworthy SM, Bloch DA, McShane DJ, Fries JF, Cooper NS, Healey LA, Kaplan SR, Liang MH, Luthra HS, Medsger TA Jr, Mitchell DM, Neustadt DH, Pinals RS, Schaller JG, Sharp JT, Wilder RL, Hunder GG. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988;31:315–324.
14. Chen M, Boilard E, Nigrovic PA, Clark P, Xu D, Fitzgerald GA, Audoly LP, Lee DM. Predominance of cyclooxygenase 1 over cyclooxygenase 2 in the generation of proinflammatory prostaglandins in autoantibody-driven K/BxN serum-transfer arthritis. Arthritis Rheum 2008;58:1354–1365.
15. Kouskoff V, Korganow AS, Duchatelle V, Degott C, Benoist C, Mathis D. Organ-specific disease provoked by systemic autoimmunity. Cell 1996;87:811–822.
16. Hoare TR, Kohane DS. Hydrogels in drug delivery: Progress and challenges. Polymer 2008;49:1993–2007.
17. Huang X, Brazel CS. On the importance and mechanisms of burst release in matrix-controlled drug delivery systems. J Controlled Release 2001;73:121–136.
18. Rothenfluh DA, Bermudez H, O'Neil CP, Hubbell JA. Biofunctional polymer nanoparticles for intra-articular targeting and retention in cartilage. Nat Mater 2008;7:248–254.
19. Vemula PK, Cruikshank GA, Karp JM, John G. Self-assembled prodrugs: An enzymatically triggered drug-delivery platform. Biomaterials 2009;30:383–393.
20. Jadhav SR, Vemula PK, Kumar R, Raghavan SR, John G. Sugar-derived phase-selective molecular gelators as model solidifiers for oil spills. Angew Chem Int Ed Engl 2010;49:7695–7698.
21. Omari A, Tabary R, Rousseau D, Calderon FL, Monteil J, Chauveteau G. Soft water-soluble microgel dispersions: Structure and rheology. J Colloid Interface Sci 2006;302:537–546.
22. Palmer MP, Abreu EL, Mastrangelo A, Murray MM. Injection temperature significantly affects in vitro and in vivo performance of collagen-platelet scaffolds. J Orthop Res 2009;27:964–971.
23. Bell CJ, Carrick LM, Katta J, Jin Z, Ingham E, Aggeli A, Boden N, Waigh TA, Fisher J. Self-assembling peptides as injectable lubricants for osteoarthritis. J Biomed Mater Res A 2006;78:236–246.
24. Shelburne KB, Pandy MG, Torry MR. Comparison of shear forces and ligament loading in the healthy and ACL-deficient knee during gait. J Biomech 2004;37:313–319.
25. Shelburne KB, Pandy MG, Anderson FC, Torry MR. Pattern of anterior cruciate ligament force in normal walking. J Biomech 2004;37:797–805.
26. Curran S, Murray GI. Matrix metalloproteinases: Molecular aspects of their roles in tumour invasion and metastasis. Eur J Cancer 2000;36:1621–1630.
27. Rooseboom M, Commandeur JNM, Vermeulen NPE. Enzyme-catalyzed activation of anticancer prodrugs. Pharmacol Rev 2004; 56:53–102.
28. Chourasia MK, Jain SK. Pharmaceutical approaches to colon targeted drug delivery systems. J Pharm Pharm Sci 2003;6:33–66.
29. Sinha VR, Kumaria R. Microbially triggered drug delivery to the colon. Eur J Pharm Sci 2003;18:3–18.