Crosslinked hyaluronic acid dermal fillers: a comparison of rheological properties

Samuel J. Falcone, Richard A. Berg
FzioMed Inc., 231 Bonetti Drive, San Luis Obispo, California 93401

Received 30 November 2006; revised 20 April 2007; accepted 31 July 2007
Published online 15 January 2008 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jbm.a.31675

Abstract: Temporary dermal fillers composed of crosslinked hyaluronic acid (XLHA) are space filling gels that are readily available in the United States and Europe. Several families of dermal fillers based on XLHA are now available and here we compare the physical and rheological properties of these fillers to the clinical effectiveness. The XLHA fillers are prepared with different crosslinkers, using HA isolated from different sources, have different particle sizes, and differ substantially in rheological properties. For these fillers, the magnitude of the complex viscosity, $|\eta^*|$, varies by a factor of 20, the magnitude of the complex rigidity modulus, $|G^*|$, and the magnitude of the complex compliance, $|J^*|$ vary by a factor of 10, the percent elasticity varies from 58% to 89.9%, and the tan δ varies from 0.11 to 0.70. The available clinical data cannot be correlated with either the oscillatory dynamic or steady flow rotational rheological properties of the various fillers. However, the clinical data appear to correlate strongly with the total concentration of XLHA in the products and to a lesser extent with percent elasticity. Hence, our data suggest the following correlation: dermal filler persistence = [polymer] × [% elasticity] and the clinical persistence of a dermal filler composed of XLHA is dominated by the mass and elasticity of the material implanted. This work predicts that the development of future XLHA dermal filler formulations should focus on increasing the polymer concentration and elasticity to improve the clinical persistence. © 2008 Wiley Periodicals, Inc. J Biomed Mater Res 87A: 264–271, 2008

Key words: dermal fillers; hyaluronic acid; soft tissue augmentation; oscillatory dynamic properties

INTRODUCTION

Dermal fillers for cosmesis of the face were introduced many years ago and received a large following with the introduction of injectable bovine collagen.[1] As the procedures for wrinkle correction with dermal fillers became more popular, the search began for safer, longer lasting fillers. The next generation filler after bovine collagen was crosslinked hyaluronic acid (XLHA), which was an improvement over bovine collagen in that it did not require a skin test for hypersensitivity and appeared to be longer lasting.[2] The search for safer and more effective dermal fillers has continued because patients desire temporary fillers that are safe and predictably last longer than 6 months.[3,4] In spite of a plethora of new temporary fillers composed of XLHA that are available in Europe and are undergoing clinical testing in the United States, it is not understood what parameters control the performance of these fillers. Although several studies have demonstrated that XLHA fillers are more persistent than bovine collagen,[5] the differences among the various XLHA fillers have not demonstrated obvious improvements in performance. One recent study compared two XLHA fillers, Perlane and Hylaform,[6] and additional studies are expected as more fillers become available.[7] Comparison of dermal fillers' effectiveness is compounded by the injection techniques,[8] and the biological response of tissue to an implant in terms of degree of inflammation.[3]

Here we compare the rheological properties of several commercial XLHA dermal fillers to understand the differences between them in terms of their physical properties and to attempt to correlate physical properties with performance. One feature of hyaluronic acid (HA) that has been useful in some medical device applications is its ability to form a cohesive gel.[9] Cohesiveness is a function of concentration and molecular weight. The property of cohesiveness, although an advantage for certain applications, is generally not an advantage as a dermal filler[9] because highly cohesive (HA) materials are generally dilute solutions of noncrosslinked

Correspondence to: R. A. Berg; e-mail: raberg@fziomed.com

of the dermis and therefore must be elastic in a low-shear environment. It is hypothesized that elasticity of a dermal filler leads to increased persistence but comparisons have not been performed. For noncrosslinked HA, dynamic rheological analysis demonstrated that as the frequency decreases the elastic properties decrease and hence, at low frequencies, the material becomes less elastic, and more viscous. Therefore, HA used in dermal fillers is always crosslinked to form gel particles that have high elasticity at lower frequencies.[10] Dermal fillers prepared from XLHA are predominately elastic at low-shear environments and must have low viscosity under high shear to be able to be delivered through a small-bore needle. These unusual requirements have prompted us to compare commercially available dermal fillers prepared from XLHA in terms of their rheological properties.

Clinical data comparing different XLHA dermal fillers are only starting to become available in the literature. This study was undertaken to compare the physical properties of currently marketed XLHA fillers to determine which physical properties of XLHA dermal fillers are responsible for effectiveness when injected intradermally for soft tissue augmentation. Since the clinical data directly comparing commercial fillers to each other in the same study are not available and most studies have compared a given filler to bovine collagen in nasolabial folds in a split-face design, we have confined our clinical data set to using the wrinkle severity rating scale (WSRS) scoring system for fillers used in clinical studies reported to the FDA.[11]

MATERIALS AND METHODS

Materials

The dermal fillers were obtained from commercial sources. Restylane, Restylane SubQ, Restylane Perlane, Restylane Touch, and Restylane LIPP were obtained from Q-Med AB, (Uppsala, Sweden), or Medicis, (Scottsdale, AZ). Hylaform, Hylaform Plus, Juvederm 24, Juvederm 24HV, Juvederm 30, and Juvederm 30HV were obtained from Allergan (Inamed) (Irvine, CA) or LEA Derm (Paris, France). Puragen was obtained from Mentor (Edinburgh, UK), and Esthelis Basic was obtained from Anteis S.A (Geneva, Switzerland).

Rheological measurements

Small deformation oscillation dynamic rheological measurements were carried out with a Thermo Haake RS300 Rheometer, Newington, NH, fitted in the cone and plate geometry. All measurements were performed with a 35-mm/1° titanium cone sensor at 25°C. Oscillation measurements were carried out at 1 Hz (6.28 rad/s). Percent elasticity is calculated as: Percent elasticity $= (100 \times G')/(G' + G'')$.[10,12]

RESULTS

The HA dermal filler formulations evaluated in this study are all crosslinked. The exact nature of the crosslinking reaction conditions, crosslinker type, crosslink density, resultant particle size, and particle shape, all affect the physical properties of the XLHA formulation. While noncrosslinked HA forms a viscous solution when dissolved in aqueous solvents, chemically XLHA produces a material that swells in aqueous solution but does not dissolve. Hence, the XLHA dermal fillers are not solutions of XLHA but suspensions of swollen XLHA particles in aqueous solution. The properties of the XLHA products are influenced by the XLHA particle size and amount of polymer per unit volume. These materials do not have a smooth appearance and depending on the injection technique used can be lumpy. Also, suspensions of crosslinked polymers require larger gauge needles for injection into the dermis compared with the solutions of noncrosslinked polymers.

Table I lists some properties of several commercially available dermal fillers containing XLHA. The source of HA is from bacterial fermentation except for the Hylaform products where the source of HA is animal (Avian). The crosslinkers used include vinyl sulfone, for the Hylaform products, 1,4-butanediol diglycidyl ether (BDDE), for the Restylane and Juvederm series as well as Esthelis Basic. Puragen is crosslinked with 1,2,7,8-diepoxyoctane (DEO). Much has been written concerning the nature of the crosslinking reactions of XLHA dermal fillers. The differences in the products from different manufacturers are generally ascribed to the physical state of the swollen gel after crosslinking HA. Products are described as being single or double crosslinked; particulate or nonparticulate; monophasic, or biphasic.

The Restylane, Juvederm, and Esthelis Basic products all use BDDE as the crosslinking agent for HA. In the Restylane series, the crosslinking reaction produces particles of crosslinked HA that are swollen in the aqueous phase. The number of particles/mL and the size of the particles differentiate the products. As the size of the particles increases the number of particles/mL decreases, and the products are advertised for use in the correction of deeper facial defects. The number of particles/mL for some of the Restylane family of products is listed in Table I.

The crosslinking reaction for the Juvederm family of products is a patented process that produces a single phase, nonparticulate, crosslinked HA gel according to the manufacturer. This crosslinking

TABLE I
The Physical Properties of XL HA Dermal Fillers

Product	Manufacturer	Concentration (mg/mL)	HA Source	Crosslinker	No. of Particles/mL	Indication (EU) CE Mark	Needle Size (ga)
ylaform	Inamed	4.5–6.0	Avian	Vinyl sulfone		Moderate to severe wrinkles	30
ylaform Plus	Inamed	4.5–6.0	Avian	Vinyl sulfone		Moderate to severe wrinkles	27
estylane Touch[a]	Q-Med AB	20	Bacteria	BDDE	500,000	Superficial lines	30
estylane[a]	Q-Med AB	20	Bacteria	BDDE	100,000	Moderate to severe wrinkles	30
estylane Perlane[a]	Q-Med AB	20	Bacteria	BDDE	10,000	Deep dermis and lips	27
estylane SubQ[a]	Q-Med AB	20	Bacteria	BDDE	1000	SubQ, add volume	Lg. bore
estylane LIPP[a]	Q-Med AB	20	Bacteria	BDDE	ND	Lips	27
ıvederm 24	LEA Derm	24	Bacteria	BDDE	ND	Medium to deep wrinkles	27
ıvederm 24HV	LEA Derm	24	Bacteria	BDDE	ND	Mid dermis and lips	27
ıvederm 30	LEA Derm	24	Bacteria	BDDE	ND	Mid dermis and lips	30
ıvederm 30HV	LEA Derm	24	Bacteria	BDDE	ND	Medium to deep wrinkles and lips	27
sthelis Basic	Anteis	25	Bacteria	BDDE, CPM	ND	Mid or deep dermis and lips	27
uragen	Mentor			DEO, double crosslinked		Mid dermis and lips	27

[a]Q-med product literature.
BDDE: 1,4 butanediol diglycidyl ether, CPM: cohesive polydensified matrix technology results in monophasic double crosslinked HA, DOE: 1,2,7,8-diepoxyoctane—ouble crosslinked—both ether and ester links formed during crosslinking reaction, ND: not determined.

Juvederm a softer feel when injected into the dermis and good persistence without the stiffness of other XLHA dermal fillers. Esthelis Basic products use a proprietary technology, cohesive polydensified matrix (CPM) to produce a single-phase nonparticulate XLHA dermal filler according to the manufacturer. Puragen uses DEO in a double crosslinking reaction that results in both ether and ester bonds crosslinking HA chains. According to the manufacturer, this highly crosslinked material is expected to improve the persistence of the Puragen products. The concentrations of XLHA in these formulations varies from ~ 5 mg/mL to 24 mg/mL. Increasing the concentration of XLHA above ~ 25 mg/mL becomes problematic because the products become too difficult to be injected through a small-bore needle.

Table II lists some rheological properties, at 0.628 (rad/s), of several commercially available dermal fillers containing XLHA. The magnitude of the complex viscosity ($|\eta^*|$), at 0.628 (rad/s) of the dermal filler formulations, Figure 1, varies widely from 58 to 1199 Pa s, almost 20 fold. Restylane LIPP has the highest magnitude of complex viscosity, 1199 Pa s and Esthelis Basic, using the CPM technology, the lowest. The XLHA products have a wide range of complex viscosities at low frequency.

The magnitude of the complex viscosity, $|\eta^*|$, for the Restylane family of products varies from 330 Pa s for Restylane SubQ to 1199 Pa s for Restylane LIPP. For the Restylane family of products, the rheological properties could be affected by the number of XLHA particles contained per milliliter in the product. The rheological properties of Restylane SubQ, Restylane Perlane, Restylane, and Restylane Touch were measured and the results indicate that although the number of particles/mL changes and the products all have different particle sizes, and indications for use of these products are different, Table I, the magnitude of the complex viscosities for all of these products are similar. Restylane Touch has 500,000 particles and a magnitude of complex viscosity of 422.5 Pa s, Restylane has 100,000 particles/mL and a magnitude of complex viscosity of 532.4 Pa s, and Restylane Perlane has 10,000 particle/mL with a magnitude of complex viscosity of 486.4 Pa s. The $|\eta^*|$, at 0.628 (rad/s), for these Restylane family of products does not relate to the number of XLHA particles contained per milliliter. Figure 2 demonstrates that for the Restylane family products, Perlane, Restylane and Restylane Touch, all have similar percent elasticity that is not a function of the number of particles/mL. A change in number of particles/mL from 10^6 to 10^4 per mL is not associated with a significant change in the percent elasticity indicating that the percent elasticity for the product is independent of number of par-

TABLE II
The Rheological Properties, at 0.628 (rad/s) of HA-Based Dermal Fillers

Product	$\lvert\eta^*\rvert$ (Pa s)	$\lvert G^*\rvert$ (Pa)	$\lvert J^*\rvert$ (1/Pa)	tan (δ)	% Elasticity
Puragen	941.8	591.7	0.0017	0.24	80.4
Hylaform	136.4	85.7	0.0117	0.14	88.0
Hylaform Plus	108.2	68.0	0.0147	0.11	89.9
Restylane LIPP	1199.0	753.5	0.0013	0.18	84.9
Restylane	532.4	334.5	0.0030	0.28	78.2
Restylane Perlane	486.4	305.6	0.0033	0.30	77.2
Restylane Touch	422.5	265.5	0.0038	0.32	75.6
Restylane SubQ	330.4	207.6	0.0048	0.39	71.8
Juvederm 30HV	81.89	51.46	0.01943	0.27	78.7
Juvederm 24HV	151.5	95.2	0.0105	0.31	76.2
Juvederm 30	93.9	59.0	0.0170	0.35	74.1
Juvederm 24	58.4	36.7	0.0272	0.53	65.2
Esthelis Basic	61.6	38.7	0.0258	0.70	58.8

For the Juvederm family of products, the $\lvert\eta^*\rvert$, at 0.628 (rad/s), varies from 152 to 58 Pa s, Figure 1. The magnitude of the low frequency complex viscosity increases for Juvederm 24, to Juvederm 30HV, next is Juvederm 30, with Juvederm 24HV having the highest magnitude of $\lvert\eta^*\rvert$, and the products are intended for different indications. However, Juvederm 24HV and Juvederm 30HV are stated to have higher viscosities and perceived to be the most persistent of the Juvederm products even though the magnitude of the complex viscosity, $\lvert\eta^*\rvert$, of Juvederm 30HV is no higher than Juvederm 30. Although the magnitude of the complex viscosity of Juvederm 24 HV is higher than Juvederm 24, the magnitude of the complex viscosity,$\lvert\eta^*\rvert$ at 0.628 (rad/s), are all below 200 Pa s for this family of products. Again, the magnitude of the low frequency complex viscosity does not seem to correlate to persistence or indicated use for the Juvederm family of products.

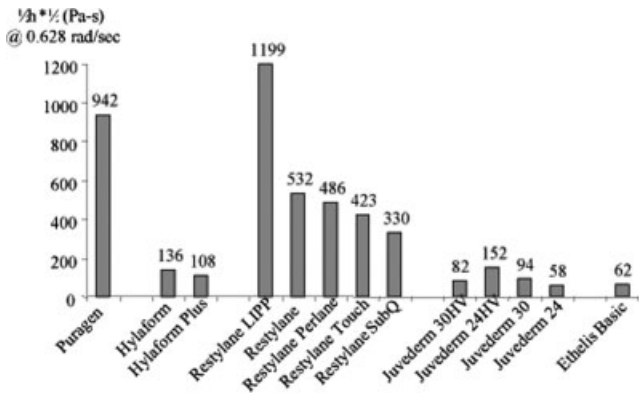

Figure 1. A plot of the magnitude of the complex viscosity, $\lvert\eta^*\rvert$ (Pa s), at 0.628 (rad/s) for the XLHA dermal fillers listed in Table II. The magnitude of $\lvert\eta^*\rvert$ varies widely from 1199 to 58 Pa s for these products. Puragen and the Restylane series have the highest magnitudes of complex viscosities while Hylaform, Juvederm, and Esthelis Basic

The magnitude of the complex rigidity modulus, $\lvert G^*\rvert$, at low frequency, 0.628 (rad/s), for all products is listed in Table II. The magnitude of $\lvert G^*\rvert$ at low frequency relates to the overall stiffness of the dermal filler at low deformation rate. The magnitude of the complex rigidity modulus, $\lvert G^*\rvert$, versus frequency, for the dermal fillers is shown in Figure 3, and the higher the magnitude of the complex modulus, the stiffer the material. There is a large range, ~ 10-fold, in the magnitude of the complex modulus versus frequency response of the XLHA dermal fillers studied here. Puragen has the highest magnitude of stiffness and Juvederm 24 or Esthelis Basic has the lowest magnitude of stiffness. The Restylane family of products has higher magnitudes of complex modulus than the Juvederm family of products. For the Restylane and Juvederm product families,

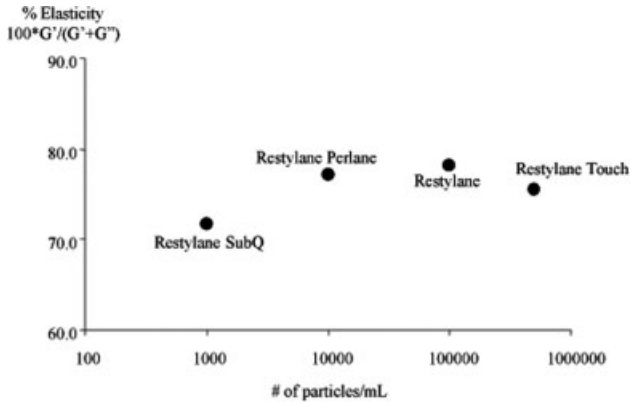

Figure 2. A plot of the percent elasticity at 0.628 (rad/s), $(100 \times G'/(G' + G''))$, versus the number of particles contained per milliliter for Restylane Touch, Restylane, Restylane Perlane, and Restylane SubQ. The data indicate that there is no correlation between the percent elasticity and the number of particles/mL and hence, the particle size in this Restylane product series. For this Restylane family of products, the number of particles/mL do not correlate to any of the low frequency (0.628 rad/s) rheological parame-

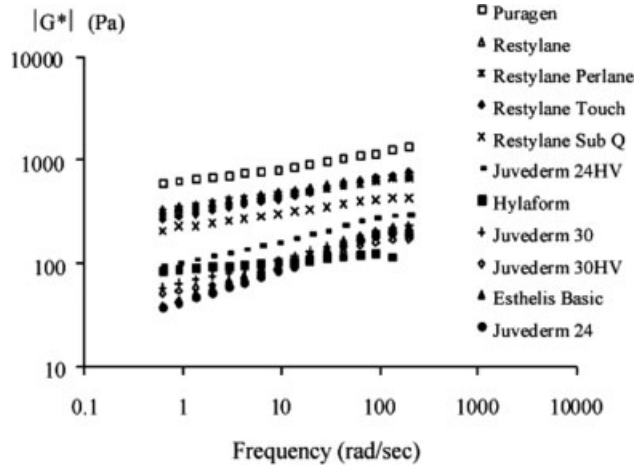

Figure 3. A plot of the magnitude of complex modulus, $|G^*|$ (Pa), versus frequency for the dermal filler listed in Table II. In this figure, Hylaform Plus and Restylane LIPP have been omitted. In this figure, the legend order is in descending magnitude of $|G^*|$ at 0.628 (rad/s). Puragen has the highest magnitude of $|G^*|$at 0.628 (rad/s) and Juvederm 24 has the lowest magnitude of $|G^*|$at 0.628 rad/s. For this set of products, the magnitude of $|G^*|$ varies over 10 fold from the stiffest material, Puragen, to the least stiff, Juvederm 24. The magnitude of the modulus or overall stiffness for these products is probably due to the crosslink density. It is also of interest to note that although they have different magnitudes, the slope of the $|G^*|$ versus frequency curves is very similar for all of the XLHA products described here. This is not surprising since the structure of the crosslinked polymer swollen in the matrix is very similar for all XLHAs.

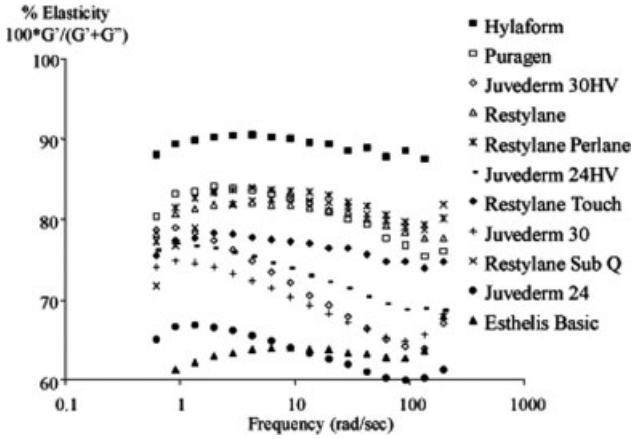

Figure 4. A plot of the percent elasticity, $(100 \times G'/(G' + G''))$, versus frequency of the dermal fillers listed in Table II. In this figure, Hylaform Plus and Restylane LIPP have been omitted and the legend order is in descending percent elasticity at 0.628 (rad/s). Hylaform has the highest percent elasticity at 0.628 (rad/s) and Esthelis Basic has the lowest percent elasticity at 0.628 (rad/s). The percent elasticity for these XLHA products varies widely from ~ 60 to 90%. Since Hylaform has the highest percent elasticity but the lowest 6-month improvement WSRS scores, (see Fig. 6), percent elasticity of the XLHA dermal filler itself does not correlate to product persistence and concentration must be taken into effect.

the magnitude of the complex rigidity modulus is more similar within each family than between families.

Several studies concerning XLHA products have referred to a rheological property called the percent elasticity.[10,12,13] In these studies percent elasticity is calculated as $(100 \times G')/(G' + G'')$ and is reported as the proportion of elasticity in an XLHA formulation. The percent elasticity versus frequency, for the XLHA dermal filler materials, is shown in Figure 4. The XLHAs have percent elasticity that range from 60 to 90% with the Restylane series more closely grouped than the others. There is a considerable range in the percent elasticity of the XLHA products with Juvederm 24 and Esthelis being the least elastic and Hylaform the most elastic.

The magnitude of the complex compliance, $|J^*|$, versus frequency, for these materials, is shown in Figure 5. The compliance is the inverse of the modulus, and is a measure of how easy it is to deform a material. Again, the data indicate that the XLHA dermal fillers have a wide range of magnitudes of complex compliance of over 10 fold. Puragen has the lowest magnitude of complex compliance of all the

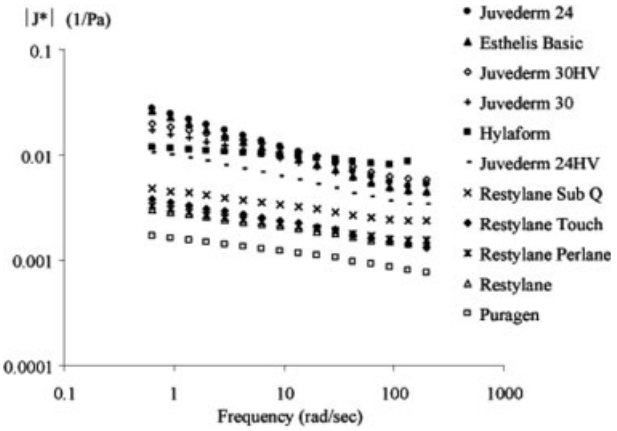

Figure 5. A plot of the magnitude of the complex compliance, $|J^*|$ (1/Pa), versus frequency of the dermal fillers listed in Table II. In this figure, Hylaform Plus and Restylane LIPP have been omitted. The magnitude of $|J^*|$ is a measure of the overall ease of deformation of a material and hence, a material with a lower magnitude of complex compliance is harder to deform than a material with a higher magnitude of complex compliance. In this figure, the legend order is in descending complex compliance at 0.628 (rad/s). Puragen has the lowest compliance and is the most difficult to deform, at 0.628 (rad/s). Juvederm 24 has the highest magnitude of $|J^*|$ and is easiest to deform, at 0.628 (rad/s). The magnitude of $|J^*|$ for these XLHA products varies widely (~ 10 fold). Puragen is the least compliant dermal filler followed by the Restylane products all of which have a magnitude of $|J^*|$ below 0.01. The remaining products all have a magnitude of $|J^*|$ above

DOCKET ALARM

Explore Litigation Insights

Docket Alarm provides insights to develop a more informed litigation strategy and the peace of mind of knowing you're on top of things.

Real-Time Litigation Alerts

Keep your litigation team up-to-date with **real-time alerts** and advanced team management tools built for the enterprise, all while greatly reducing PACER spend.

Our comprehensive service means we can handle Federal, State, and Administrative courts across the country.

Advanced Docket Research

With over 230 million records, Docket Alarm's cloud-native docket research platform finds what other services can't. Coverage includes Federal, State, plus PTAB, TTAB, ITC and NLRB decisions, all in one place.

Identify arguments that have been successful in the past with full text, pinpoint searching. Link to case law cited within any court document via Fastcase.

Analytics At Your Fingertips

Learn what happened the last time a particular judge, opposing counsel or company faced cases similar to yours.

Advanced out-of-the-box PTAB and TTAB analytics are always at your fingertips.

API

Docket Alarm offers a powerful API (application programming interface) to developers that want to integrate case filings into their apps.

LAW FIRMS
Build custom dashboards for your attorneys and clients with live data direct from the court.

Automate many repetitive legal tasks like conflict checks, document management, and marketing.

FINANCIAL INSTITUTIONS
Litigation and bankruptcy checks for companies and debtors.

E-DISCOVERY AND LEGAL VENDORS
Sync your system to PACER to automate legal marketing.