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Filling the Gap: A Correlation between Objective and Subjective Measures of Injectability

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Various injectable biomaterials are developed for the minimally invasive delivery of therapeutics. Typically, a mechanical tester is used to ascertain the force required to inject these biomaterials through a given syringe-needle system. However, currently there is no method to correlate the force measured in the laboratory to the perceived effort required to perform that injection by the end user. In this article, the injection force (F) for a variety of biomaterials, displaying a range of rheological properties, is compared with the effort scores from a 50 person panel study. The maximum injection force measured at cross-head speed 1 mm s^{-1} is a good proxy for injection effort, with an R^2 of 0.89. This correlation leads to the following conclusions: participants can easily inject 5 mL of substance for $F < 12$ N; considerable effort is required to inject 5 mL for 12 N < F < 38 N; great effort is required and <5 mL can be injected for 38 N < F < 64 N; and materials are entirely non-injectable for $F > 64$ N. These values may be used by developers of injectable biomaterials to make decisions about formulations and needle sizes early in the translational process.

1. Introduction

Injectable therapeutic systems are a rapidly growing sector of the biomaterials field, with applications ranging from ophthalmology to orthopedics, cosmetics to cancer treatment.[1] These formulations offer benefits over traditional injection of actives in aqueous solutions, since the biomaterial system can impart structural support, act as a scaffold for cell driven regeneration, and localize as well as sustain drug release. Injection is advantageous primarily because it is minimally invasive, reducing the pain, scarring, and infection risk associated with implantation.[2] In addition, injected materials are usually fluid prior to application. This allows any additives, such as drugs or cells, to simply be mixed in before administration, and the injected material can conform perfectly to the tissue surface.[3,4]

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Other than niche applications, such as shape memory alloys for wound closure and small electronics for diagnosis and signaling, injectable biomaterials generally fall into two categories: cements and polymers.[1] An exception to this dichotomy is polymethyl methacrylate (PMMA), a polymeric cement. Biomedical cements are solutions or slurries that set in the body; therefore, they are typically used for hard tissue applications in orthopedics and dentistry. Many cements precipitate to form calcium salts, including sulphates, silicates, and various phosphates; however, PMMA and bioglasses are also common materials.[5]

Calcium sulphate hemihydrate (CS), for example, has been used since the 1890s to fill bone defects as it is osteoconductive, non-toxic, biodegradable, and does not illicit an immune response.[6–8] It sets when mixed with water because it hydrates to form insoluble gypsum crystals:[9,10]

$$CaSO_4.\frac{1}{2}H_2O + \frac{3}{2}H_2O \rightarrow CaSO_4.2H_2O \quad (1)$$

These cements are studied and used for bone grafting,[11–14] delivery of antibiotics,[15–18] and are often combined with polymers or other ceramics to improve handling, material, and biological properties. Commercial CS-based cements are widely available, and include Cerament (Bone Support AB, Sweden), DentoGen (Orthogen Corporation, USA) and OsteoCure (Futura Biomedical, USA).

In contrast to cements, injectable polymeric biomaterials may be used as viscous solutions, hydrogels, microspheres, nanospheres, and films. Often having a high water content, these materials are softer than cements, and are commonly used to deliver cells and therapeutics for tissue regeneration and targeted drug delivery.[19] One of the most widely studied polymers is alginate, a natural polysaccharide derived from various brown seaweeds (*Phaeophyceae*), due to its biocompatibility, non-toxicity, mild gelation by the addition of divalent cations, and gel structure similar to the extracellular matrix.[20] For injectable applications, alginate may be used directly as a solution,[21] or microspheres.[22] Alternatively, alginate has been gelled in situ by co-injection with calcium ions[23] or a poorly soluble calcium salt that is later broken down.[24] Calcium carbonate and sulphate are often used as they display low solubility at neutral

pH, but break down when the pH is lowered, often triggered in situ by the addition of glucone-δ-lactone.[25]

Notably when reviewing the literature for studies focused on developing injectable biomaterials, many do not directly measure injectability. Focus is instead placed on rheology to identify gelation time, study post-shear recovery, and measure viscoelastic properties.[26–28] For studies which directly measure injectability, typically a mechanical tester is used to compress the plunger of a syringe at a set rate (Figure 2A), and measure the force required to extrude the biomaterial, over distance or time (Figure 2B,C). This set up has been used to assess the injectability of cements,[29–31] hydrogels,[32,33] and composites.[34,35] An alternative method of equipping a syringe with force and displacement sensors has also been utilized.[36] However, results are often compared to each other or to a standard, rather than to a force threshold below which a material is known to be injectable. For cements, injectability is often tested and defined by the percentage mass of material that remains in the syringe following an injection test. This is to measure the degree of filter pressing, a phenomenon seen in cements that occurs due to the liquid phase being expelled during setting, resulting in phase separation.[29,37] Notably, the force applied during this commonly used injectability test may vary by orders of magnitude.[3,38] Higher forces, up to 300 N, are acceptable for cements intended for use with a high-pressure injector, though for biomaterials that are intended for hand delivery the force used becomes significant. This highlights a need to understand the force that an end user can comfortably apply to inject a biomaterial through a given syringe needle system.

Usability testing is employed in several industries where the view of the end user is important to successful uptake of the product. Notable examples include sensory testing in the food and pharmaceutical industries, and simulated use of technology, including for medical devices.[39–42] Benefits of participant testing include reduction in overall cost, primarily by avoiding late stage redesign or reformulation, improved usability of the final product, and access to end user perspectives.[43] However, usability testing is often not carried out because it can take considerable time, money, and organization.[44,45]

For the first time, this study fills the gap between objective laboratory measurements and subjective effort scores of injectability. To achieve this, the force required to extrude a range of biomaterials, with varying rheological properties, through a syringe-needle system was measured using a standard mechanical tester set-up. The same formulations were also injected by 50 participants, and quantitatively evaluated using a 5-point scale of effort, ranging from easily injectable (1) to entirely non-injectable (5). Comparing these two measures of injectability allows a correlation to be established between laboratory measurements and the effort required by the end user. This allows researchers to make more informed decisions about which biomaterial formulations to take forward, and to select syringe and needle sizes early in the translational pipeline, without having to undertake initial usability studies, saving time and money.

2. Results

2.1. Material Characterization

The force required to induce a material to flow, and thus to be injected, will be influenced by its rheological properties. Alginate solutions and CS cement were used as model biomaterials for this study, in addition to water. As expected, dissolved alginate thickened the water, and viscosity increased with polymer concentration. Notably, the standing viscosity increased over four orders of magnitude between 1 and 10 w/v% polymer concentration. All alginate solutions were observed to exhibit shear thinning behavior, whereby the apparent viscosity decreases with increasing shear rate. For example, the 10 w/v% solution viscosity decreased by three orders of magnitude over the shear rates tested (**Figure 1**A). CS cement was also shear thinning, with a viscosity at a shear rate of 0.1 s^{-1} 85% lower than at 1 s^{-1}. The test was carried out for 90 s, to simulate the time over which the injection would take place, and to prevent damage caused by bulk setting between the rheometer plates. However, an increase in cement viscosity of on average 400% can still be observed over the 90 s studied (Figure 1B).

2.2. Objective Injectability Evaluation

The objective injectability of each biomaterial-needle combination was determined using a mechanical tester to measure the force required to compress the plunger at a set rate (**Figure 2**A). Typical force-extrusion curves display an initial gradient as the plunger is compressed and the biomaterial is accelerated. This behavior continues until a peak (maximum

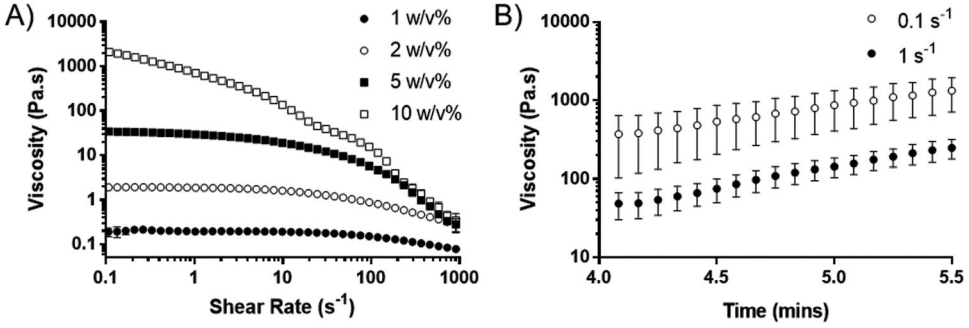


Figure 1. Rheological characterization of tested materials. A) Shear-ramps showing the shear-thinning behavior of alginate solutions, whose standing viscosity increases with polymer concentration. B) Peak holds at shear rates of 0.1 and 1 s^{-1} showing the time-dependent viscosity and the shear-thinning behavior of CS cement. Mean ± SD, $n = 3$.

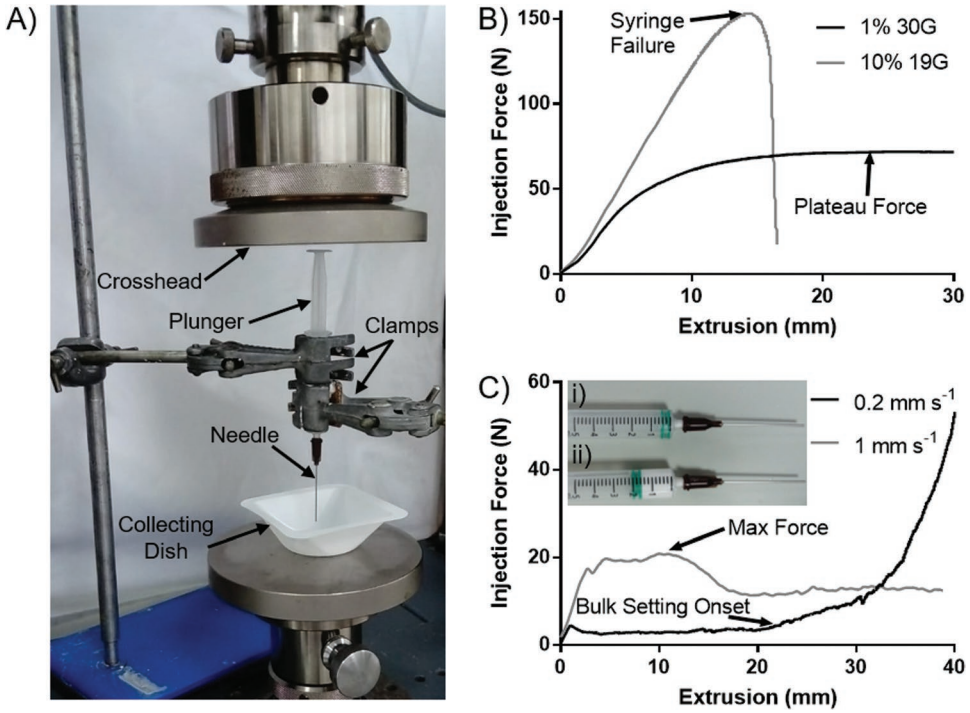


Figure 2. Objective injectability measurement by extrusion analysis. A) Set-up for objective injectability analysis: the loaded syringe-needle system is statically held with a series of clamps and the crosshead is set to push down on the plunger at a set rate, and force required to extrude the biomaterial is recorded. B) Typical injection force curves for a system with no pre-plateau force peak (gray line, 1 w/v% alginate, 30G needle, 1 mm s^{-1}), and a system in which the syringe-needle system fails prior to a plateau (dark gray line, 10 w/v% alginate, 19G needle, 1 mm s^{-1}). C) Typical injection force curves for calcium sulphate cement which has (black line), and has not (gray line), set in situ, the latter displaying a maximum force peak. Insert displaying photograph of syringe needle systems following extrusion testing, showing i) full expulsion of the cement through the syringe needle, ii) bulk cement setting preventing full expulsion.

force) is reached, which is followed by a plateau (plateau force) that occurs when the extrusion rate of the biomaterial reaches a constant speed (Figure 2C, gray line). However, no maximum force peak was observed when the plateau force was greater than the force required to move the plunger and accelerate the biomaterial (Figure 2B, black line). As such, a peak was only seen for lower viscosity samples passing through a large needle. For samples that required a larger amount of force, greater than 130 N, the plunger would deform and break before the extrusion could reach a plateau (Figure 2B, gray line).

CS cement, which sets in situ, only displayed a plateau when the cement was fully extruded prior to solidification (Figure 2C, gray line, insert i). Compared to the alginate solutions, the cement force–displacement data was less smooth, likely due to heterogeneous setting throughout the syringe leading to differences in crystal size and thus a higher degree of variability. Once the cement began to set in bulk some filter pressing was observed, which lead to expulsion of the liquid phase (water), and the solid system was compressed until the plunger failed (Figure 2C, black line, insert ii).

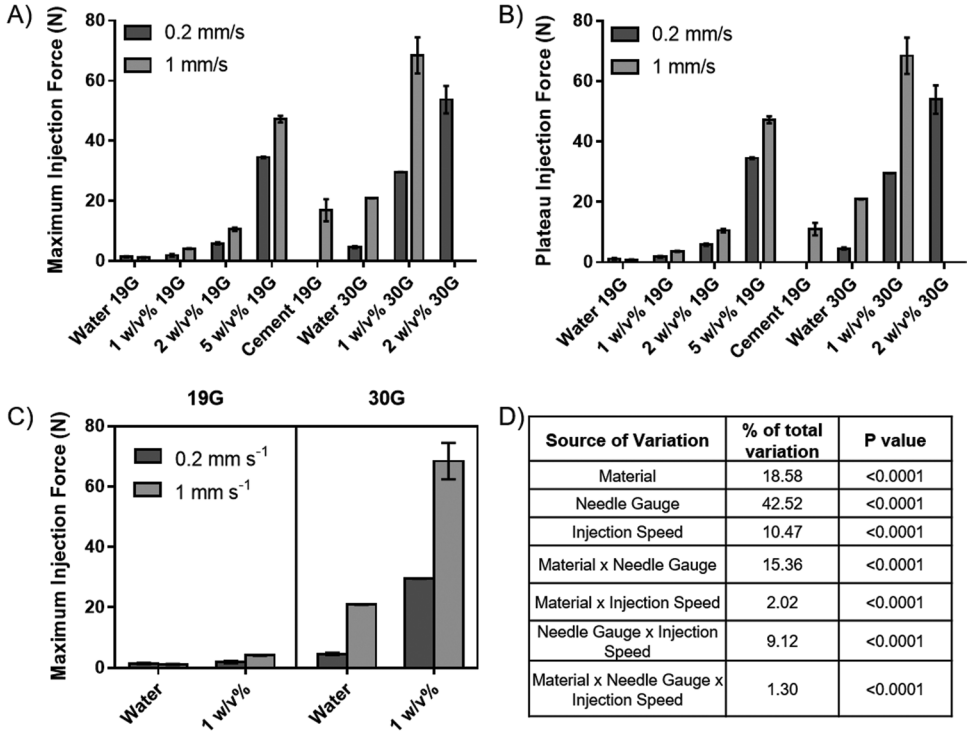
Two key values can be extracted from the force injection curves; maximum and plateau force. However, only water, 1 w/v% alginate and CS cement, when expelled from a 19G needle, displayed a distinctive maximum force peak. For the other biomaterial-needle combinations, the maximum and plateau force were equivalent. Biomaterial-needle combinations that could not be injected (10 w/v% alginate for both needles, and 5 w/v% alginate and CS cement through the 30G needle) are not shown. For all non-injectable systems, failure occurred at 142 ± 11 N, suggesting that this is a property of the syringe rather than the biomaterial inside.

The force required for injection increased with increasing polymer concentration (from water through the alginate samples), increasing injection speed, and higher needle gauge (decreased needle diameter) (**Figure 3**A,B). The plateau force for 2 w/v% alginate was similar to that for the CS cement.

A 3-way ANOVA was performed on water and 1% alginate, which were the only biomaterials where the injection force could be determined for both compression rates and needle sizes (Figure 3C). This revealed that material, compression rate and needle size all had a significant effect on the required injection force ($p < 0.001$), with needle size having the largest effect (Figure 3D). There was a significant interaction between all three variables.

2.3. Subjective Injectability Evaluation

Subjective injectability effort was established by a participant study, where 50 individuals were asked to rate how difficult they found it to inject each biomaterial through each needle (**Figure 4**A). Participants found it more difficult to inject alginate solutions with a higher concentration, and to inject



Source of Variation	% of total variation	P value
Material	18.58	<0.0001
Needle Gauge	42.52	<0.0001
Injection Speed	10.47	<0.0001
Material x Needle Gauge	15.36	<0.0001
Material x Injection Speed	2.02	<0.0001
Needle Gauge x Injection Speed	9.12	<0.0001
Material x Needle Gauge x Injection Speed	1.30	<0.0001

Figure 3. Objective force measurements. A) The maximum force required to extrude water, calcium sulphate cement, and 1, 2, and 5 w/v% alginate from the syringe-needle system. B) The plateau force required to extrude the biomaterials from the syringe needle system. C) 3-way plot showing the interaction of material, needle gauge, and injection speed on maximum injection force, for data where all three are available (water and 1 w/v% alginate). D) Summary of 3-way ANOVA on maximum injection force, for data where all three variables were obtained. Mean ± SD ($n = 3$).

biomaterials through a smaller needle (Figure 4B). Material-needle combinations not shown (cement, and 5 and 10 w/v% alginate through a 30G needle) scored a 5, and thus were entirely non-injectable, from all 50 participants.

A 3-way ANOVA was performed on water, and 1 and 2 w/v% alginate, which were at least partially injectable for both injection rates and needle sizes (Figure 4C). The majority of the variation came from the needle gauge, followed by the material, and there was no significant difference between male and female participants (Figure 4D). However, a significant interaction was found between material and needle gauge.

2.4. Correlation between Objective and Subjective Measures of Injectability

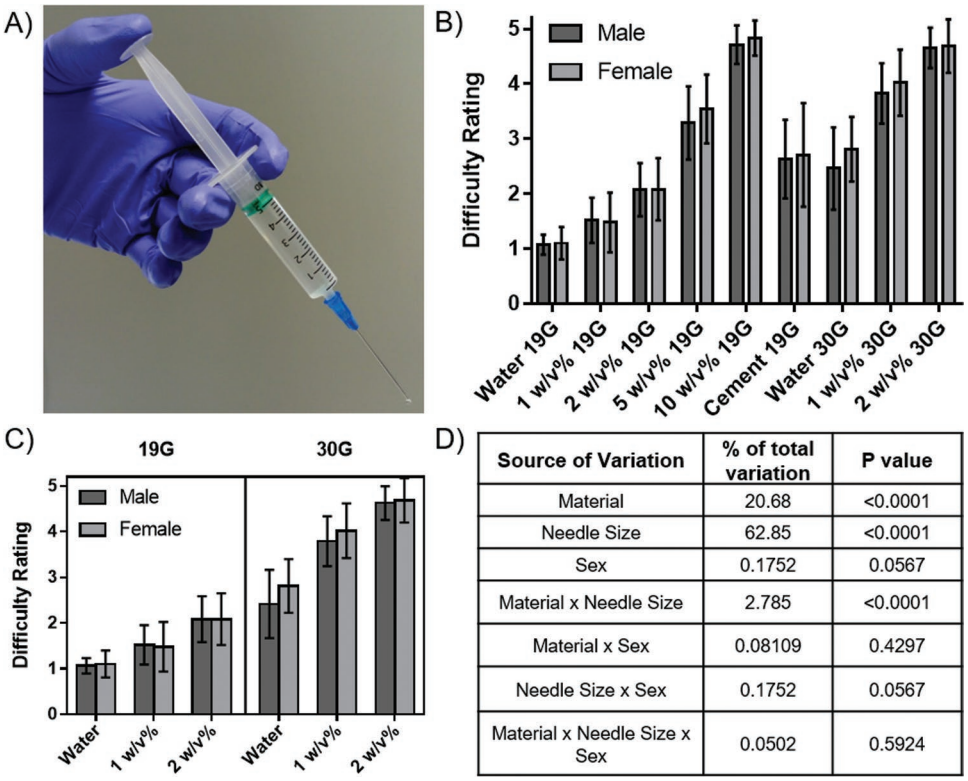
By plotting the objective injection force from the extrusion study against the subjective injection force from the participant study, the correlation between them can be determined using a linear regression.

The strongest correlation with effort score was found using the maximum injection force at 1 mm s^{-1}, with an R^2 value of 0.89 (**Figure 5**A). This correlation is less strong for the plateau force, with an R^2 value of 0.85 (Figure 5B). Compared to the maximum force at 1 mm s^{-1}, the correlation is not as strong when relating effort score with the 0.2 mm s^{-1} injection speed, with an R^2 value of 0.87 (Figure 5C,D). The two data points that appear to be abnormally high (black arrows) are for water and 1 w/v% alginate with a 30G needle.

The participant effort score can thus be calculated from the maximum injection force found at 1 mm s^{-1} using this linear correlation (**Figure 6**). Further, this correlation can also be used to define boundaries for injectability regimes. Participants can easily inject 5 mL for forces less than 12 N while considerable effort is required for forces between 12 and 38 N. Further, great effort is required and less than 5 mL can be injected for forces between 38 and 64 N, while materials are entirely non-injectable for forces greater than 64 N.

3. Discussion

Injections are becoming increasingly popular as minimally invasive ways to deliver biomaterials for regenerative medicine or targeted delivery of drugs.[46,47] Unlike traditional injections, which have the physical properties of water, biomaterials are typically more viscous or semi-solid when injected. Following injection, they may solidify further to form scaffolds for tissue regeneration or depots to locally deliver sustained concentrations of a drug to a target site. To enable translation of promising formulations, it is important to develop biomaterials that may be easily handled and applied by the end user. This may affect choices of syringe and needle dimensions, particularly as a smaller needle may be required in animal models compared to human trials. Aligning characterization data of injectable biomaterials in clinically relevant application devices with an assessment of usability would help inform important decisions that need to be made during translation. Ultimately,



Source of Variation	% of total variation	P value
Material	20.68	<0.0001
Needle Size	62.85	<0.0001
Sex	0.1752	0.0567
Material x Needle Size	2.785	<0.0001
Material x Sex	0.08109	0.4297
Needle Size x Sex	0.1752	0.0567
Material x Needle Size x Sex	0.0502	0.5924

Figure 4. Subjective assessment of injectability. A) Ergonomic position in which participants held the syringe for subjective injectability analysis. B) Subjective difficulty rating given by participants to extrude the biomaterial from the syringe needle system. C) 3-way plot showing the interaction of material, needle gauge and sex on maximum injection force, for data where all three are available (water, 1 and 2 w/v% alginate). D) Summary of 3-way ANOVA on maximum injection force, where all three variables are available. Mean ± SD (n = 24–26).

considering and addressing delivery early in this pathway will determine whether a developed biomaterial is likely to be accepted into common usage, as clinicians are less likely to adopt new products they find difficult to use.

In this study, water, alginate solutions, and CS cement were used as representative examples of common biomedical materials, displaying a range of rheological properties. Water is a Newtonian fluid, with a viscosity of 0.001 Pa s at 20 °C.[48] The viscosity of alginate solutions was found to increase non-linearly with polymer concentration (Figure 1A), as the chains order the water, overlap and entangle.[49,50] Furthermore, these solutions displayed shear-thinning behavior, whereby the apparent viscosity decreased as the applied shear increases. This is because at low shear the entanglements between polymers are broken at the same rate as they are made. However, at higher shear rates the entanglements are broken more quickly than they are made. Thus, the network breaks down and the polymer chains align in the shear field, which reduces viscosity.[51,52] Alginate solutions are known to be pseudoplastic, which means that their viscosity is dependent on shear but independent of time.[53,54] CS cements were also found to be shear thinning (Figure 1B); however, their rheological properties are also dependent on time. Further, they exhibit a yield stress which must be overcome in order to break the interparticle network and initiate flow.[55,56] This network is formed of colloidal interactions between particles, and the CS dihydrate forming at the intersection of flocculated particles, giving the cements some elastic character. CS cements are thus thixotropic, as the viscosity, yield stress and elastic modulus increase with time, but are decreased by shear in a time-dependent manner.[57] The rheological characteristics of CS cement are thus not only dependent on the applied shear, but also the shear history, and their viscoelastic and time-dependent nature means that several rheological measurements are needed to fully characterize them.[57–59]

A standard mechanical testing set-up (Figure 2A) was used to find the force required to compress the syringe plunger and extrude each biomaterial from a 5 mL syringe, through a 19G or 30G needle, at 0.2 or 1 mm s^{-1}. Mechanical testing apparatus is common in research institutions, and is already commonly used for injectability testing.[29–35] Mechanical testing can be performed with any biomaterial and syringe-needle system, and was found to be highly reproducible for the formulations tested in this study (Figure 3A,B). Of the two key values that can be extracted from injection curves, maximum force is arguably more objective and can quickly be identified computationally. Determination of the plateau force requires some user input to identify its starting point. However, measuring the average force at the plateau may be a better approach for samples, such as cements, which generate relatively "noisy" data (Figure 2C). The higher degree of localized force–displacement variation may be due to the range of particle sizes in the cement. During setting both the hemi- and dihydrate forms of calcium sulphate are present, and dihydrate crystal growth and aggregation may not be uniform.[10] As particulates of different sizes pass through the

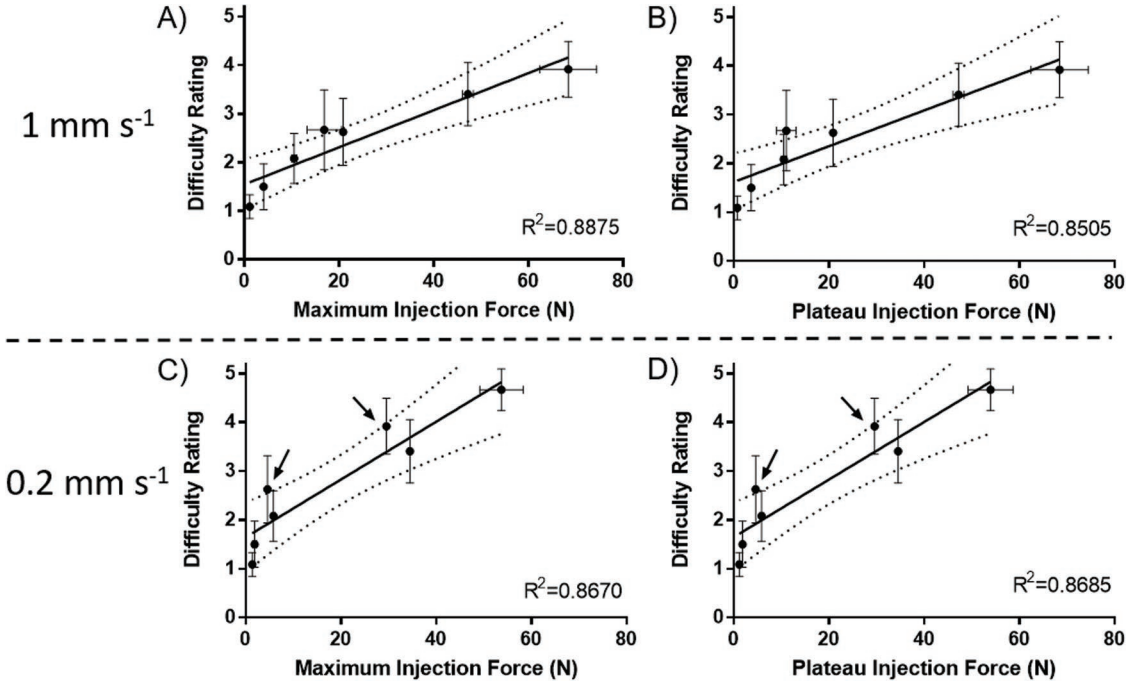


Figure 5. Correlation between objective and subjective measures of injectability. A) Correlation between maximum injection force and difficulty rating at 1 mm s^{-1}. B) Correlation between plateau injection force and difficulty rating at 1 mm s^{-1}. C) Correlation between maximum injection force and difficulty rating at 0.2 mm s^{-1}. D) Correlation between plateau injection force and difficulty rating at 0.2 mm s^{-1}. Points are mean ± SD ($n = 3$ for force, $n = 50$ for difficulty rating). Line is linear regression ± 95% confidence bands, with displayed R^2 value.

needle, the force will slightly increase to different degrees, and then decrease once it has passed, appearing as localized variations in the force–displacement curve (Figure 2C). Nevertheless, a maximum force peak is only seen for samples where the force to move the plunger is greater than that to extrude the biomaterial. This only occurred for easily injectable samples, thus for the majority of samples where the injectability is in doubt, the maximum and plateau force values are the same.

The force required for injection generally increases with biomaterial viscosity, speed of injection, and needle gauge (Figure 3). The exception to this is CS cement that, if injected too slowly, solidifies in situ and becomes non-injectable. By considering the needle as a pipe, these results can be contextualized with a version of the Hagen–Poiseuille equation,[60]

$$F = \frac{8R_s^2 LQ\eta}{R_n^4} + F_f \tag{2}$$

where F is the injection force (N), R_s is the internal syringe radius (m), R_n is the internal needle radius (m), L is length (m), Q is fluid flow rate ($m^3\ s^{-1}$), η is the dynamic viscosity (Pa s) and F_f is the friction force between the plunger and barrel wall (N). Increasing polymer concentration increases the viscosity (Figure 1A), resulting in a larger force required for extrusion. The set injection speed dictates the fluid flow rate through in the needle, a higher value of which increases the injection force. A higher gauge needle has a shorter length but a greater length to radius ratio, and thus the injection force increases. This also explains the significant interaction between these variables (Figure 3D), as the product of functions of viscosity, injection speed, needle length, and radius give the required force, and therefore they do not influence the force independently of each other. Equation (2) is a simplified model for Newtonian fluids such as water; the expression is even more complex for non-Newtonian fluids like shear thinning alginate solutions,[61]

$$F = 2^{n+2}\,\pi^{1-n}\,L\,R_s^2\,KQ^n R_n^{-(3n+1)}\left(\frac{3n+1}{2n+1}\right)^{n-1} + F_f \tag{3}$$

where n is the power index (-) and K is the consistency index (Pa s^n) from the Ostwald de Waele expression to describe

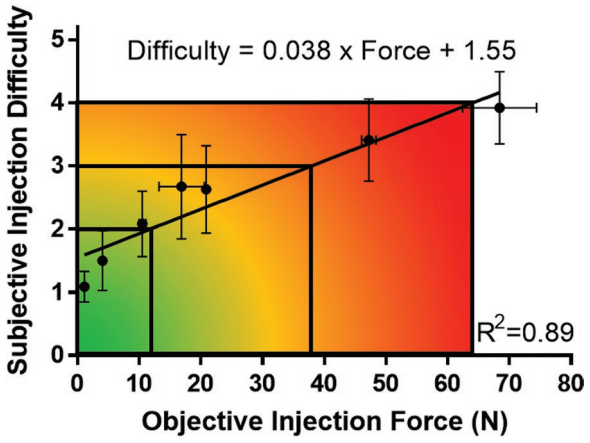


Figure 6. Boundaries of injectability regimes. The linear equation which describes the relationship between maximum injection force found at 1 mm s^{-1}, and effort score, and the injectability regime boundaries.

non-Newtonian fluid viscosity: $\eta = K\dot{\gamma}^{n-1}$, where $\dot{\gamma}$ is the shear rate (s^{-1}). Thus, if the rheological properties of such materials are known, the force can be calculated and compared to end-user effort. However, this is more complicated still for biomaterials such as in situ setting cements, with highly complex rheological characteristics. This complexity is demonstrated by the fact that CS cements display a similar injection force requirement to 2 w/v% alginate solutions (Figure 3A,B), but have a far greater measured viscosity (around 100 Pa s compared to 2 Pa s at a shear rate of 1 s^{-1}) (Figure 1). Given that such systems display thixotropy, the most accurate measure of end user injectability is obtained by mixing the cement and loading it into the syringe as would occur in clinical practice. This enables its shear history to be replicated realistically prior to testing. Notably, mechanical extrusion testing takes a short time to perform (50 s to inject 5 mL at 1 mm s^{-1}), and is more directly relatable than rotational rheology (typically 5–10 min per sample), which may also not be able to reach the shear rate experienced in a narrow needle. For example, the shear rate when injecting at 1 mm s^{-1} through a 30G needle is around 166000 s^{-1},[62] whereas material is typically ejected from a rotational rheometer at speeds greater than 1000 s^{-1}.

The same biomaterials, in the same syringe-needle systems, were given to 50 participants, who rated the effort required to perform the injection on a 5-point scale (Figure 4). This is a relatively large number of participants; a sensory panel typically only has 3–15 people.[63] A great number of participants is required in order to find a meaningful correlation, given the relatively large variability seen in effort score (maximum SD of 1 effort score unit) (Figure 4B). Given that effort score correlated well with injection force (R^2 of 0.89), it is likely that a large part of this variation comes from disparity in hand strength. However, interestingly there was not a significant difference in effort score between male and female participants (Figure 4D), despite men generally displaying greater hand strength.[64] It may be that the size and shape of the syringe prevents participants with larger or stronger hands applying greater force. A future study examining the relationship between hand strength and perceived injection effort is required to elucidate this further.

The effort score from the participant study shows the same trend as the objective extrusion force; increased effort is required as biomaterial viscosity and needle gauge increase. Further, the proportion of this trend contributed by each variable is similar for both studies, with the majority of variation coming from the needle gauge, followed by the material (Figures 3D and 4D). This suggests that injection force, rather than duration, energy or power for example, is a good proxy for perceived effort, and provides a causative explanation as to why the two values correlate well. While the R^2 values for correlations at both test speeds and force values (maximum or plateau) are similar, the strongest correlation is seen when comparing effort score to the maximum force at 1 mm s^{-1} with an R^2 of 0.89. This suggests that maximum force is the limiting factor for injectability. However, as discussed earlier, the maximum and plateau force is the same for the majority of samples. This further suggests that participants were injecting, on average, at 1 mm s^{-1}, and therefore that this is the optimum test speed. When correlating the effort score with the extrusion forces at 0.2 mm s^{-1}, biomaterials injected through the narrower 30G needle received comparatively high effort scores (Figure 5C,D, arrows). This indicates that participants perceived a higher effort to inject through the narrower needle, for a comparable measured force requirement, than the wider. Given that participants likely injected slower through the narrower needle, this suggests that duration may increase perceived effort, in addition to force. Clearly, duration is also important when considering biomaterials with a time-dependent viscosity, such as cements.

The amount of biomaterial to be injected should be taken into consideration when interpreting these correlations. Injection volumes of the order of 1–10 mL, as studied here, may be realistic for some applications like orthopedics.[65] However, in the case of fields like ophthalmology, volumes less than 100 µL may be used.[66] For small animal studies, which are common preclinical stages in the biomaterial translational pipeline, volumes may be 5 µL or less.[67] In these cases, one might expect a higher effort score may be allowable, for only a short duration. On the other hand, for such precision applications, a low effort may be necessary to maintain accuracy. The type of syringe will also change the force required. Force decreases with a narrower syringe (Equation (2)), and thus injecting the same material through the same needle will, for example, require less effort with a 1 mL syringe compared to the 5 mL syringes used in this study. Holding the syringe in a different ergonomic position may also alter the force that can be applied. A limitation of this study's methodology is that injecting biomaterial into open air is not entirely representative of injecting material into tissues. However, a previous study found a consistent 10% increase in force between injecting into soft tissue and open air.[68] This may be easily taken into account prior to evaluating the effort score.

4. Conclusion

The force required to extrude a range of common biomaterials, exhibiting varying rheological properties, was correlated to the effort required to inject these biomaterials by 50 users. Comparing these values allowed, for the first time, a correlation to be drawn between objective injection force and subjective injection effort, with an R^2 of 0.89. This correlation applies for a wide range of material properties and needle gauges, and shows that participants can easily inject 5 mL for $F < 12$ N; considerable effort is required to inject 5 mL for 12 N $< F <$ 38 N; great effort is required and less than 5 mL can be injected for 38 N $< F <$ 64 N; and materials are entirely non-injectable for $F >$ 64 N. Overall, the findings herein support researchers making key decisions about biomaterial formulations and syringe-needle dimensions early in the translation pipeline, without the requirement for time consuming and potentially expensive end-user studies.

5. Experimental Section

Materials: Deionized water was obtained from a Milli-Q system (Millipore). Alginic acid sodium salt from brown algae, medium viscosity (alginate) was purchased from Sigma, and calcium sulphate hemihydrate

>97% pure from Acros Organics. 5 mL disposable syringes (SKU 307731, Beckton Dickenson) were attached to 19G (SKU: NB19G1.5, internal diameter 0.69 mm, length 38 mm) or 30G (SKU: NB30G0.5, internal diameter 0.16 mm, length 13 mm) blunt needles (NeedlEZ). These needle gauges were chosen to give a large range, and to represent typical sizes used in small animal and large animal or human applications. 5 mL of fluid was used for extrusion and participant testing.

Alginate solutions (1, 2, 5 and 10 w/v%) were prepared by dissolving in deionized water at room temperature. Water and alginate solutions of 1 and 2 w/v% were aspirated into syringes, 5 and 10 w/v% were too viscous and were top loaded into syringes. Solutions were used after 24 h, to remove transient viscosity changes, and negate the need for antimicrobial agents. CS (4 g) in deionized water (5 mL) was manually mixed for 1 min until homogeneous. For extrusion and participant testing, cement was top-loaded into the syringe, and testing began 4 min after the water was added. This was to ensure consistency, and enough time to load the syringe into the mechanical tester and begin the test. All material preparation and testing were carried out at 20 °C.

Rheology: Rheometric testing was carried out on a Kinexus Ultra+ rheometer (Malvern). Alginate solutions were tested using a 40 mm, 4° angle cone, and plate geometry, and the shear rate was continuously ramped from 0.1 to 1000 s^{-1}, over 10 min. CS cements were spooned onto the rheometer plate and tested using a 40 mm parallel plate, with a gap of 1 mm. The viscosity was measured at a constant shear rate, starting at 4 min after the water was added. Testing of cements was carried out for 90 s, to replicate the time period in which injection would be carried out. The test was stopped after this period to prevent the cement setting between the plates.

Extrusion Testing: Objective mechanical testing was carried out on a Z030 universal mechanical tester (Zwick Roell), equipped with a 50 kN load cell. The syringe was suspended securely with a system of clamps, the plunger was compressed at a set rate, and the force required to expel the formulation through the needle was recorded (Figure 1). Two injection speeds were selected, 0.2 mm s^{-1} and 1 mm s^{-1}, to represent a slow and fast injection, respectively.

Participant Study: Approval for this study was granted by the STEM Ethical Review Committee at the University of Birmingham. 50 participants (26 male, 24 female) between the ages of 18 and 28 were recruited for the study. Participants were asked to hold the syringe in their dominant hand, with index and middle finger under the pommel with their thumb over the plunger (Figure 4A). Each participant was asked to attempt to inject the entirety of each syringe. Participants were asked to rate how difficult they found each injection on a scale of 1 to 5, defined as:

1. Minimal effort required to inject.
2. Some effort required to inject.
3. A lot of effort required to inject.
4. Maximal effort required —could not inject the entire 5 mL.
5. Not injectable —could not inject any of the material.

Participants were uninformed of the contents of each syringe, and each participant was given each material-needle combination in a random order to minimize intra-participant comparison.

Statistical Testing: All graphs show mean ± standard deviation (SD). Results were considered significant for $p < 0.05$. 3-way ANOVAs were only performed on data sets where results for all variables were available. For the participant study, two male individuals' data was removed at random to give the equal participant numbers required for the 3-way ANOVA. All tests are two-tailed. Statistical tests were performed in Prism 7 (GraphPad).

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

alginate, biomaterials, cement, injectability, testing methods

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