International Journal of Pharmaceutics 546 (2018) 272–278

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Improved delivery of PLGA microparticles and microparticle-cell scaffolds in clinical needle gauges using modified viscosity formulations

Omar Qutachi[a], Emma J. Wright[a,b], Gemma Bray[a], Omar A. Hamid[a], Felicity R.A.J. Rose[a], Kevin M. Shakesheff[a], Derfogail Delcassian[a,c,*]

[a] Division of Regenerative Medicine and Cellular Therapies, Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, NG7 2RD, UK
[b] School of Pharmacy, University of Lincoln, LN6 7DL, UK
[c] David H. Koch Institute for Integrative Cancer Research, MIT, 02140 MA, USA

ARTICLE INFO

Keywords:
High viscosity formulation
Microparticle delivery
Cell particle scaffolds
Needle gauge

ABSTRACT

Polymer microparticles are widely used as acellular drug delivery platforms in regenerative medicine, and have emerging potential as cellular scaffolds for therapeutic cell delivery. In the clinic, PLGA microparticles are typically administered intramuscularly or subcutaneously, with the clinician and clinical application site determining the precise needle gauge used for delivery. Here, we explored the role of needle diameter in microparticle delivery yield, and develop a modified viscosity formulation to improve microparticle delivery across a range of clinically relevant needle diameters. We have identified an optimal biocompatible formulation containing 0.25% pluronic F127 and 0.25% carboxymethyl cellulose, which can increase delivery payload to 520% across needle gauges 21–30G, and note that needle diameter impacts delivery efficacy. We use this formulation to increase the delivery yield of PLGA microparticles, and separately, PLGA-cell scaffolds supporting viable mesenchymal stem cells (MSCs), demonstrating the first *in vitro* delivery of this cell scaffold system. Together, these results highlight an optimal formulation for the delivery of microparticle and microparticle-cell scaffolds, and illustrate how careful choice of delivery formulation and needle size can dramatically impact delivery payload.

1. Introduction

Poly (DL-lactic acid-co-glycolic acid) (PLGA) materials are widely used therapeutics with applications in drug delivery (Rao et al., 2010; Rafati et al., 2012; Simon-Yarza et al., 2013), tissue engineering (Park et al., 2016), and cellular scaffolding (Lee et al., 2015; Qutachi et al., 2014). In drug delivery applications, PLGA microparticles offer tunable, biodegradable kinetic release profiles. They are FDA approved for a variety of applications, and can often be administered *via* localised or systemic injection (Rafati et al., 2012; Cappellano et al., 2014; Park et al., 2014). Larger 3-dimensional PLGA structures have also been surgically implanted as cellular scaffolds for regenerative medicine (Hernandez et al., 2010; Kochenderfer et al., 2010), however there are limited examples of systems combining the extracellular support matrix provided by PLGA (Lee et al., 2015; Han et al., 2015) with the tunable kinetic release of soluble factors (Cappellano et al., 2014). Through this combination of a physical support matrix and soluble cellular cues, microparticle cell scaffold systems with controlled release properties are able to support and direct transplanted cell behaviour.

A current challenge in the delivery of microparticles, cells, or microparticle-cell combination therapies lies in maintaining an effective therapeutic dose across varied application routes. In many systems, a common clinical administration route is the localised injection of materials using either pre-filled or self-filled syringes together with a needle (Garbayo et al., 2016; McHugh et al., 2015; Fu et al., 2016; Kim et al., 2016). Selection of an appropriate needle gauge depends on the therapeutic application; finer needles of 29G are often used for spatially accurate delivery of materials to the spinal cord, compared to 14G needles frequently used for intramuscular injection (Dittmann et al., 1993; Mavrogenis et al., 2015; Songur et al., 2011; Sivera et al., 2008; Raftesath and Fitzgerald, 2014). In cell-only systems, recent studies have suggested that injection parameters (including needle gauge, flow rate and applied force) can affect both the number of cells delivered and the ability of these cells to undergo phenotypic differentiation (Mamidi et al., 2012; Amer et al., 2016; Amer et al., 2015a,b), however these studies have yet to be applied to delivery of acellular microparticle systems.

Maintaining an effective therapeutic dose across a broad range of

* Corresponding author at: Division of Regenerative Medicine and Cellular Therapies, Centre for Biomolecular Sciences, School of Pharmacy, University of Nottingham, NG7 2RD, UK.
E-mail address: Derfogail.Delcassian@nottingham.ac.uk (D. Delcassian).

https://doi.org/10.1016/j.ijpharm.2018.05.025
Received 11 August 2017; Received in revised form 8 May 2018; Accepted 9 May 2018
Available online 16 May 2018

administration routes remains an ongoing consideration for the clinical application of drug delivery systems (Cilurzo et al., 2011; Kearney and Mooney, 2013). To our knowledge, there has yet to be a study on the effect of injection parameters on delivery of either PLGA microparticles alone, or PLGA microparticles in conjunction with cells. Here, we explore modified viscosity systems to enhance both microparticle delivery and microparticle-cell scaffold delivery across a range of clinically relevant needle gauges. First, we explore PLGA particle delivery across a range of needle gauges with controlled plunger force. Next, we investigate modified delivery formulations using the thickening agent carboxymethylcellulose (CMC) (Nakanishi, 1966) and the amphiphilic polymer pluronic F127 (Prameela et al., 2015), probing their ability to modify viscosity and their effect on particle delivery across needle sizes. Finally, we investigate the effect of our lead formulation on the viability of human MSCs, and demonstrate the use of our formulation in delivering multifunctional PLGA microparticle scaffolds with human MSCs *in vitro*.

2. Materials and methods

2.1. Fabrication of PLGA microparticles

Non-porous PLGA particles were fabricated using 20% PLGA (50:50, 52 kDa Lakeshore Biomaterials) in dichloromethane (DCM) (Fischer) by either a single or double emulsion method. In the single emulsion method, the polymer solution was homogenised in 250 mL of 0.3% polyvinyl alcohol (13–24 kDa, Sigma-Aldrich) using a high speed Silverson L5M homogeniser. The resulting emulsion was left stirring at 300 RPM until particles hardened. In the double emulsion method, 100 μL of an aqueous solution containing 10 mg Amoxicillin (Abcam) was homogenised in the polymer solution. The resultant primary water in oil (w/o) emulsion was then homogenised again in the 0.3% PVA and the resultant water in oil in water (w/o/w) double emulsion was left stirring until particles hardened. Particles were extracted by centrifugation, washed, and lyophilised before being stored at −20 °C until use. Porous PLGA particles were produced using a double emulsion method as previously described (Qutachi et al., 2014). Briefly, 20% (w/v) PLGA in dichloromethane was treated with phosphate buffered saline (PBS, Gibco) as a porogen. Post fabrication, the particles were treated with ethanolic sodium hydroxide (sodium hydroxide (Sigma-Aldrich) and absolute ethanol (Fischer)) to enhance surface porosity. The particles were then extracted by centrifugation, washed, and lyophilised before being stored at −20 °C until use.

Particles were characterised using scanning electron microscopy and laser diffraction. Briefly, particles were loaded onto carbon disks on aluminium stubs (Agar Scientific), sputter coated with gold (Balzers Union Ltd.) and imaged on an JEOL 6060L system. The mean diameter and particle size distribution were analysed using a Coulter LS230 particle size analyser (Beckman, UK). Particle size distribution was then determined as a function of the particle diffraction and plotted as a function of volume percentage.

2.2. Delivery formulations

Particles were resuspended at 5 mg/mL in DMEM (Gibco), containing between 0 and 10% pluronic F127 (Sigma-Aldrich) or 0–10% medium viscosity sodium carboxymethylcellulose (CMC) (Sigma-Aldrich). Combined formulations containing between 0 and 0.5% pluronic and 0–0.5% CMC were also prepared. Formulation solutions were kept at 4 °C until use. Solution viscosity was measured using a rheometer with cone and plate geometry at 0.1° angle (Anton Parr-Physica MCR 301)), using a shear ramp from 0 to 100 1/s at 25 °C.

2.3. Particle injection

PLGA microparticles (5.0 mg) were suspended in polymer/media formulation (1.0 mL) in 1.5 mL Eppendorf tubes under repeated pipetting and vortexing. The total volume was drawn up into a 1 mL disposable syringe (BD) and a needle (gauges 21G, 23G, 25G, 27G, 30G (BD Microlance)) fitted to the syringe prior to ejection of the total volume into a new Eppendorf tube. A sample (10 μL) was taken from the ejected volume and particles were counted using a haemocytometer. For comparison, particles were also ejected through needles without a syringe to provide a control. Injections were considered to have failed when the contents of the syringe could not be ejected using a mechanically controlled syringe pump. This is usually due to a blockage in the needle or aggregation of the suspension at the syringe tip, resulting in the syringe contents not being completely emptied. Injection failures are recorded and measured in counts, and the calculated values illustrates the percentage of “failed” injections per condition.

2.4. Injection forces and calculated shear rates

For each needle-syringe combination, the initial and glide force were determined using a texture analyser (TA.HD *plus*, Stable micro systems). 1 mL of formulation was loaded into a 1 mL syringe (BD), and fitted with an appropriate needle into the injection rig. A 10 mm cylinder probe was lowered into contact with the plunger, with no pre-test force, before a 1 mm/s ejection rate was applied in compression mode. The initial force was calculated as the force required to overcome the resistance to movement of the plunger, whereas the glide force was calculated as the average force required to evacuate the syringe at 1 mm/s. For formulations tested with microparticles, a concentration of 5 mg/mL particles suspended in 1 mL solution was used. Shear rates were calculated using Poiseuilles equation;

$$\gamma = \frac{4Q}{\pi r^3}$$

where γ is shear rate in s^{-1}, Q is flow rate in cm^3/s, and r is needle radius in cm. Shear rates were calculated using both experimental flow rate (for 1 mm/s plunger ejection) and theoretical flow rates 1 mL/h and 20 mL/h expected to be used in clinic, described in Table 2.

2.5. PLGA microparticle release studies

In vitro testing of the controlled release of Amoxicillin encapsulated within PLGA microparticles was performed using Transwell inserts (Corning, UK). 25 mg of PLGA microparticles were suspended in 1.5 mL of the described formulations, and incubated at 37 °C. The concentration of Amoxicillin in release medium was quantified by UV detection at 300 nm using a plate reader (Tecan) with concentration determined from a calibration curve.

2.6. Cell viability

Human bone marrow derived mesenchymal stem cells (MSCs) (UE6E7T-11 cells sourced from the Japanese Stem Cell Bank) were used for all cellular assays. The Prestoblue cell viability assay (Invitrogen Life Sciences, UK) was performed 1 and 24 h post-seeding (n = 6). Each sample was submerged in 1 mL of 10% Prestoblue (Invitrogen Life Sciences, UK) in media; all samples were incubated at 37 °C for 30 min. Triplicate 100 μL media samples from each well were read on a Tecan plate reader with the excitation wavelength set to 535 nm and the emission wavelength set at 615 nm.

2.7. Injection of cells cultured on particles

Porous particles were treated with Tween and then antibiotic/antimycotic solution (Sigma-Aldrich). Commercially available human mesenchymal stem cells (MSCs) (Japanese Stem Cell Bank) were seeded at 200,000 per well in 12-well plates, with 8 mg PLGA particles added per well, and incubated overnight at 37 °C in DMEM medium

supplemented with 10% foetal calf serum, 1% antibiotic/antimycotic solution, 1% L-glutamine (2 mM) and 1% non-essential amino acids (Sigma-Aldrich). Wells were centrifuged, and the cell pellet re-suspended in DMEM or formulation conditions. As described previously, this suspension was injected into a fresh 12-well plate, and incubated for 10 min with 10% Presto blue at 37 °C. Cell number per well was quantified using a Tecan plate reader. Samples were formalin fixed for 20 min at room temperature, washed with PBS multiple times and then imaged by SEM.

2.8. Delivery efficacy

Delivery efficacy was calculated by comparing the number of particles delivered using a specific formulation and needle combination to the number of particles delivered in a basal media solution. Particles were counted using a hemocytometer. For example, to compare the delivery efficacy of particles suspended in basal media through needle-free syringes and 27G needles, a suspension of PLGA particles was loaded into at least six identical syringes, three of which were uncapped and three capped with 27G needles. Syringes were loaded onto the controlled rate syringe pump, and ejected at constant plunger speed of 1 mm/s. An aliquot of the ejected solution was transferred to a hemocytometer and the number of ejected particles counted. To calculate delivery efficacy, we averaged the number of particles for each condition and calculated efficacy as follows;

$$delivery\ efficacy = \frac{average\ number\ of\ particles\ delivered\ in\ condition\ X}{average\ number\ of\ particles\ delivered\ in\ basal\ media} \times 100$$

2.9. Statistical analysis

Statistical analysis was performed using GraphPad Prism (Version 7) software. ANOVA analysis was used for all statistical testing, performed on data from between 3 and 6 repeat experiments. Analysis is considered significant, and the * designation is assigned, if $p > 0.05^{*}$, $p > 0.01^{**}$, $p > 0.001^{***}$, $p > 0.0001^{****}$. Bar graphs represent the mean of 3–6 individual repeats, with associated error bars to show the standard error in the mean (SEM). Details of individual statistical tests (ie. one way or two way ANOVA, number of repeats) are provided in the figure caption for each graph.

3. Results and discussion

3.1. The effect of needle gauge on microparticle delivery

We fabricated PLGA microparticles of 27 μm diameter as described in Section 2.1 (Fig. 1A), and investigated delivery efficacy through needles between 21 and 30G, corresponding to internal needle diameters currently used in clinic ranging from over 500 μm to around 160 μm (Table 1). Using a syringe pump set up with constant flow rate of 1 mm/s, we evaluated the ejection of PLGA microparticles suspended in basal media solution through a range of needle gauges (Fig. 1B). All needle gauges tested were able to deliver the microparticles, however we find that narrower needle gauges of 27G and 30G failed to deliver microparticle solutions as effectively as either needle free or large-bore needle systems, with delivery efficacy reduced to 61% in 27G needles compared to needle free systems. Given the internal needle diameters of 27G and 30G needles (210 μm and 160 μm respectively) are wider than the 26.9 μm (± SD 11.2 μm) diameter of the microparticles (Fig. 1A), this suggests that the particle delivery through narrow gauge needles is affected not only by particle size, but also the dynamics of the fluid ejection from the syringe. To explore this further, we investigated the effect of altering the solution viscosity, and so ejection fluid dynamics, of the delivery formulation in needle delivery systems.

3.2. PLGA microparticle delivery using modified formulations

Formulations containing the thickening agent carboxymethylcellulose (CMC) (Fig. 1C), pluronic F127 (Fig. 1D) to modulate wettability, and CMC/pluronic F127 (Fig. 1E) combination formulations were tested for their ability to deliver PLGA microparticles. Fig. 1 demonstrates that increasing concentrations of either CMC or pluronic F127 in media significantly increased particle delivery, illustrated by the 400% increase of particle delivery in systems using either 5% pluronic F127 or 2% CMC, compared to un-supplemented media. We then investigated combination formulations with compositions of between 0.125 and 0.5% of CMC and pluronic F127 in media. Fig. 1E illustrates that particle delivery was significantly improved in all combination formulations tested, and broadly increased as the concentration of CMC/pluronic F127 in solution increased.

We calculated the shear rate used during our experimental injections at a constant plunger rate of 1 mm/s (Table 2). These values suggest our experimental injection system experiences low shear between 150 and 500/s. We examined the effect of formulation composition on viscosity at comparable shear rates between 1 and 100/s. Comparing the formulations, Fig. 1F illustrates that the viscosity of each composition remains broadly constant against increasing shear between 1 and 100/s. In systems with constant CMC concentration and varying pluronic F127 concentration, viscosity measurements are similar, whilst viscosity measurements roughly double as the concentration of CMC doubles in the formulation, suggesting that viscosity properties are broadly driven by the CMC concentration in solution (Voigt et al., 2012; Ahmed et al., 2011). Given that many clinically administered injections are delivered at higher flow rates than those tested experimentally (Nguyen et al., 2002; Salman et al., 2017), we next calculated the expected flow rate and shear rate during more rapid administration through various needle gauges (Table 2). Needles injecting flow rates of 1 mL/h and 20 mL/h (flow rates more commonly used for clinical infusion regimes) experience much higher shear rates through similar needle gauges.

At the shear rates tested here, supplementing basal media with pluronic acid and CMC can increase microparticle delivery. There are several potential explanations for this, including the changes in solution viscosity and the increased wetting of microparticles in these formulations. The increased solution viscosity may help to form a stable microparticle suspension during syringe evacuation, which prevents particles being forced to the side of the syringe. Particles at the side of syringe can be statically attracted to the syringe casing and are less likely to be ejected (Whitaker et al., 2011). Additionally, Fig. 1E indicates that increasing the total polymer concentration to above 0.5%, can increase delivery payload. It is likely that the negatively charged polymers help to combat static and attractive charges between the particles and the syringe, facilitating particle ejection (Park et al., 2014; Voigt et al., 2012). The combined effects of increased polymer concentration, particle wetting and increased viscosity, are likely responsible for the increase in delivery.

3.3. Modified delivery formulations using clinical needle gauges

All combination formulations tested demonstrated enhanced microparticle delivery compared to a basal media control in a 23G needle system. We then explored whether these combination formulations were able to enhance microparticle delivery across a range of clinically relevant needle gauges (Dittmann et al., 1993; Mavrogenis et al., 2015; Songur et al., 2011; Raftesath and Fitzgerald, 2014; Gill and Prausnitz, 2007; Fateh et al., 2014). Fig. 2A compares the number of microparticles delivered for each formulation in 21–27G needles, and Fig. 2B summarises the average delivery across all needle gauges tested. There is a significant increase in particle delivery across all needle gauges using CMC and pluronic F127 formulations in comparison to basal media ($p < 0.001$ in all formulations, and all needle gauges, Turkey's

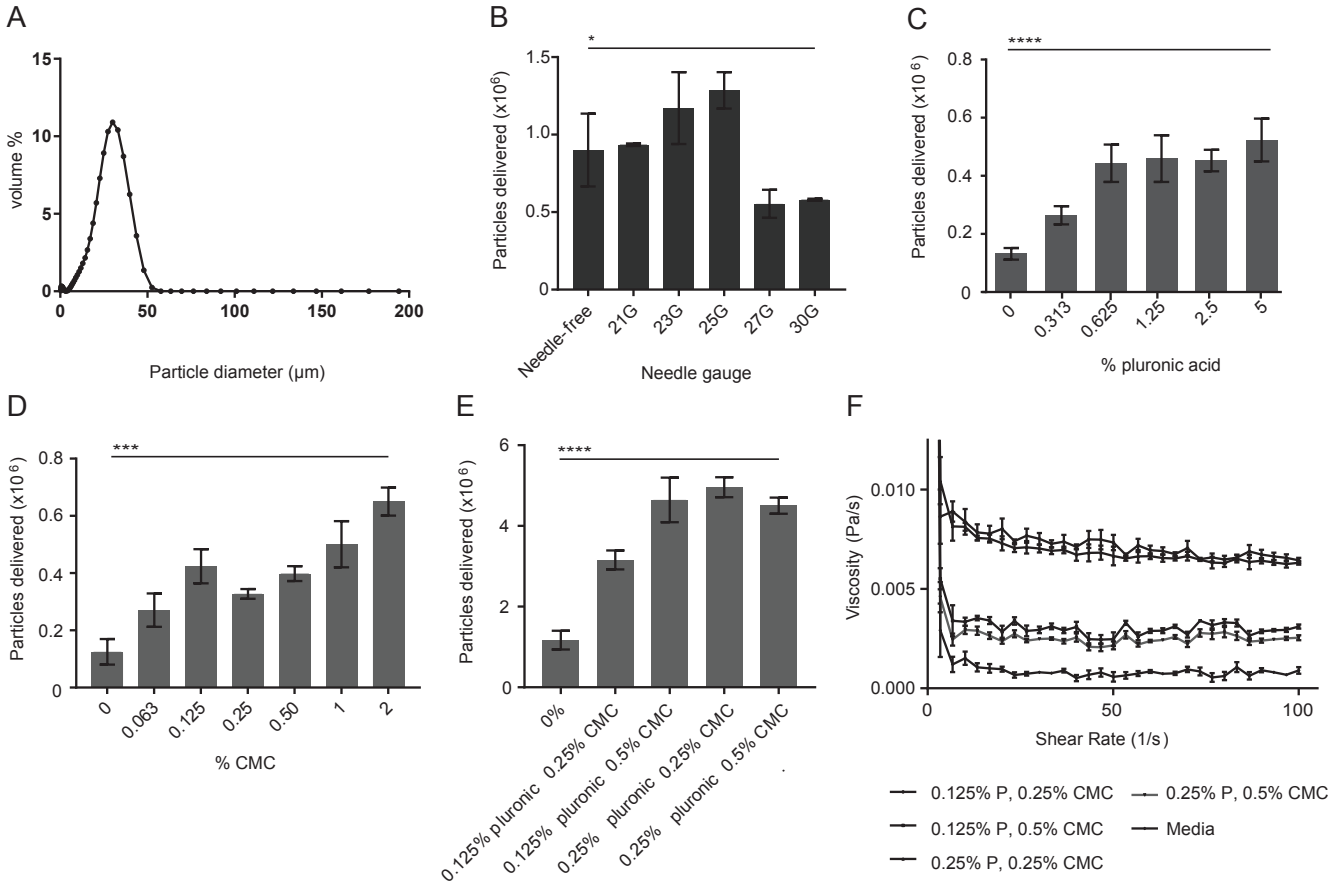

Fig. 1. Properties of pluronic F127 and CMC modified solutions. (A) Size distribution of PLGA microparticles used in this study, measured by laser diffraction. (B) PLGA microparticle delivery from a stock of 5 mg/mL through syringes fitted with various needle gauges (C–E) Increasing concentrations of pluronic F127 (C) CMC (D) and pluronic F127/CMC combination formulations (E) were tested for their ability to deliver microparticles from a stock solution through 27G needles (F) Viscosity measurements at increasing shear rate, measured for combination formulations at 25 °C. All statistical tests show one way ANOVA where $p > 0.05^{*}$, $p > 0.01^{**}$, $p > 0.001^{***}$, $p > 0.0001^{****}$, bars represent mean of 3–6 repeats with SEM error bars.

Table 1
Clinical needle gauges and needle bore internal diameters.

Needle Gauge	Needle bore diameter (mm)	Needle Gauge	Needle bore diameter (mm)
15	1.372	25	0.260
16	1.194	26	0.260
17	1.067	27	0.210
18	0.838	28	0.184
19	0.686	29	0.184
20	0.603	30	0.159
21	0.514	31	0.133
22	0.413	32	0.108
23	0.337	33	0.108
24	0.311	34	0.0826

multiple comparison tests and two-way ANOVA), with delivery increasing on average between 300 and 400% compared to particles delivered in basal media in the same needle gauge.

We further explored needle blockage and injection failure for each of these formulations (Fig. 2C) in needle gauges. Fig. 2C demonstrates that the 0.25% pluronic F127 and 0.25% CMC formulation had no injection failures compared to other formulations which occasionally resulted in a blocked needle injection failure. Considering this 0.25% pluronic F127 and 0.25% CMC formulation in more detail, we find that microparticle delivery is significantly increased in all needle gauges tested using this formulation compared to a basal media control (Fig. 2D), with increases in delivery between 320 and 750% compared to basal media (Table 3), and an average increase in microparticle delivery of 520% across all needle gauges. This formulation is therefore broadly applicable to a range of clinical needle gauges.

Table 2
Calculated shear rate through clinical needle gauges at varying flow. Shear rate was calculated using Poiseuilles equation for needle gauges between 21 and 30G, using experimental flow rates tested using 1 mm/s plunger speed, and theoretical flow rates of 1 or 20 mL/h, all values rounded to 3 significant figures.

Needle gauge	Needle diameter (cm)	Experimental flow (plunger at 1 mm/s)		Theoretical flow (1 mL/h)	Theoretical flow (20 mL/h)
		Q (cm^3/s)	Shear rate (s^{-1})	Shear rate (s^{-1})	Shear rate (s^{-1})
30G	0.159	1.98E−07	503	704,000	14,100,000
27G	0.210	3.46E−07	380	306,000	6,110,000
25G	0.260	5.31E−07	308	161,000	3,220,000
23G	0.337	8.92E−07	237	74,000	1,480,000
21G	0.514	2.07E−06	156	20,800	417,000

3.4. Modified viscosity formulations for the delivery of drug loaded microparticles

We investigated the applicability of the lead 0.25% CMC 0.25% pluronic F127 formulation for the delivery of drug-eluting microparticles and microparticle-cell scaffold therapeutics. First, the release

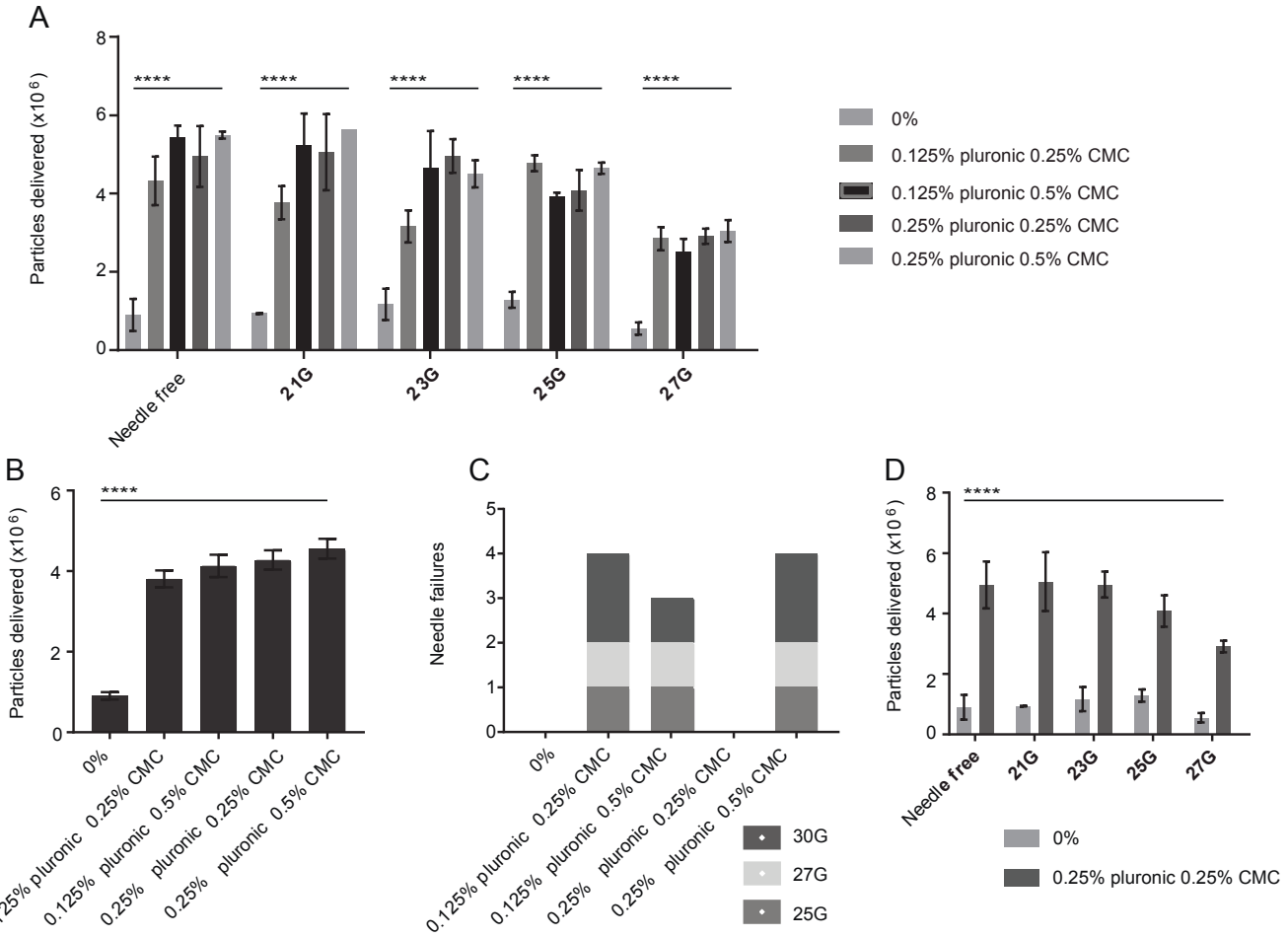

Fig. 2. Modified solutions across needle gauges. Increasing concentrations of (A) pluronic F127/CMC combination formulations were tested for their ability to deliver microparticles from a stock solution across a range of needle gauges. (B) Average delivery of microparticles across all needle gauges (21–27G) in different combination formulations. (C) Number of needle blockages in each formulation, n = 10 for each formulation. Needles 30G, 27G, 25G, 23G and 21G were tested, with blockages found in 25–30G. (D) A comparison between basal media and 0.25% pluronic 0.25% CMC for the delivery of microparticles across needle gauges 21G–27G. All statistical tests show two way ANOVA where $p > 0.05^{*}$, $p > 0.01^{**}$, $p > 0.001^{***}$, $p > 0.0001^{****}$, bars represent mean of 3–6 repeats with SEM error bars.

Table 3
Delivery efficacy Delivery efficacy was calculated by comparing the number of particles delivered. For example, PLGA microparticles were suspended in either basal media, or media supplemented with 0.25% CMC and 0.25% Pluronic F127, loaded into a standard syringe fitted with the appropriate gauge needle. Syringes were loaded onto the mechanically controlled syringe pump, and ejected. An aliquot of the ejected solution was transferred to a hemocytometer and the number of ejected particles counted. At least three syringe ejections were tested for each condition, and delivery efficacy calculated by comparing to the number of particles ejected in the basal media formulation.

Needle system and media composition	Average number of particles delivered/mL	Compare to: Needle system and media composition	Average number of particles delivered/mL	Calculated delivery efficacy (%)
Needle free, basal media	0.90×10^6	Needle free, 0.25% CMC 0.25% pluronic F127	4.95×10^6	550%
21G, basal media	0.94×10^6	21G, 0.25% CMC 0.25% pluronic F127	5.06×10^6	538%
23G, basal media	1.17×10^6	23G, 0.25% CMC 0.25% pluronic F127	4.96×10^6	424%
25G, basal media	1.29×10^6	25G, 0.25% CMC 0.25% pluronic F127	4.08×10^6	316%
27G, basal media	0.55×10^6	27G, 0.25% CMC 0.25% pluronic F127	2.91×10^6	529%
30G, basal media	0.58×10^6	30G, 0.25% CMC 0.25% pluronic F127	4.37×10^6	753%

profile of amoxicillin from amoxicillin loaded PLGA microparticles was tested, exploring microparticles delivered through a 27G needle with and without the modified viscosity formulation (Fig. 3A). As expected, the total amount of amoxicillin released was increased when our delivery formulation was used. As the kinetics of release remained broadly unaffected, we suggest that this is due to the increased delivery yield using the 0.25% CMC 0.25% pluronic F127 formulation.

Interestingly, the addition of pluronic acid and CMC may also alter microparticle surface wetting and shear forces experienced during ejection, which could also influence drug release kinetics. The hydrophilicity of PLGA surfaces affects polymer degradation kinetics, and is correlated with both co-polymer composition and liquid solvent polarity (Chen et al., 2017; Vargha-Butler et al., 2001). The addition of CMC and pluronic acid to the basal media formulation may alter the polarity of the liquid phase, and could directly impact microparticle surface wetting and so drug release kinetics. Formulations containing CMC and pluronic acid have also been shown to alter shear forces experienced by cells during stirred culture (Gallardo Rodriguez et al.,

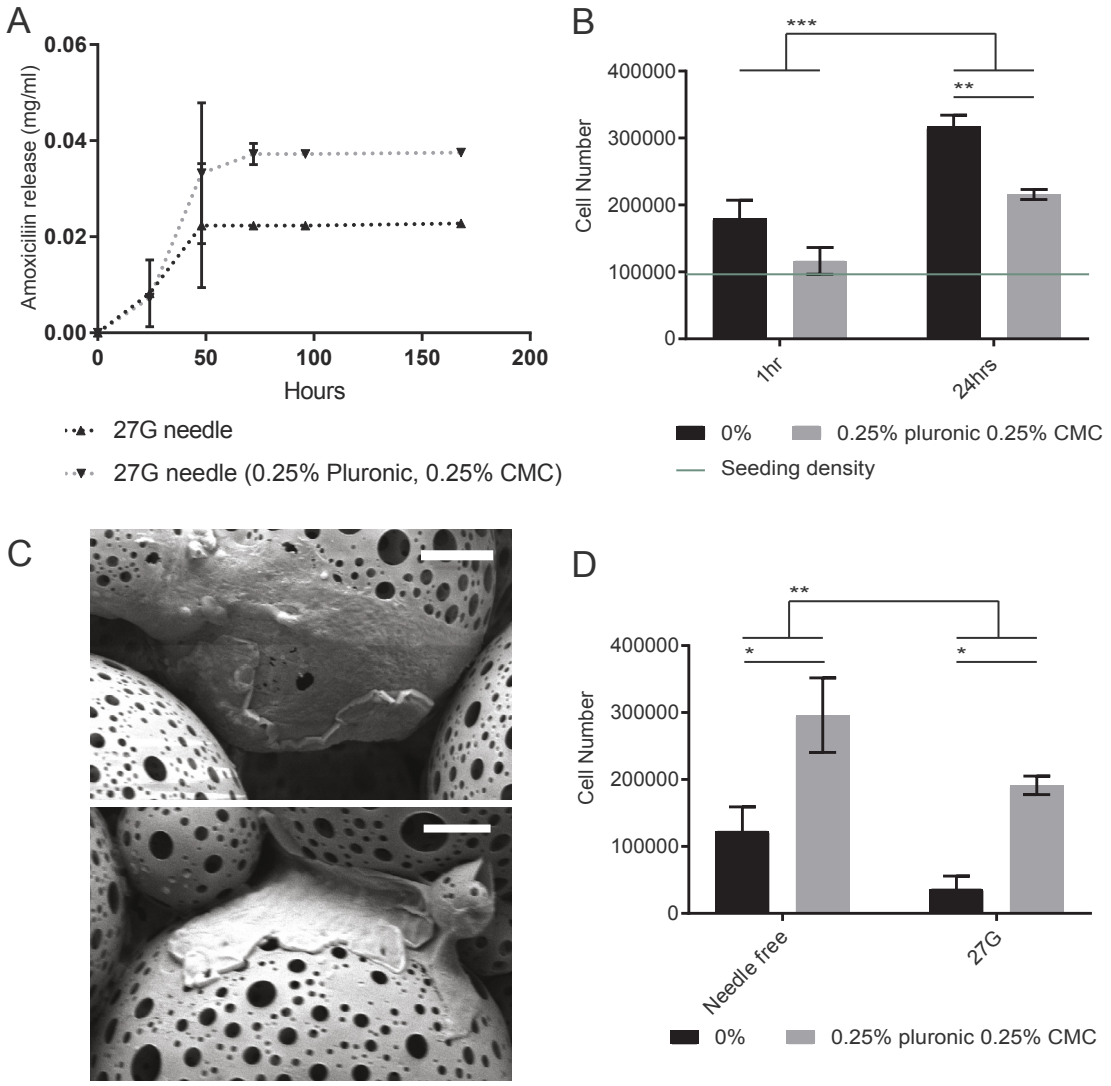

Fig. 3. Effect of modified formulations on drug release and cell behaviour (A) Cumulative amoxicillin release was analysed from PLGA microparticles delivery using syringes fitted 27G needles. PLGA microparticles were suspended in basal media or 0.25% pluronic 0.25% CMC supplemented media (C) SEM image of microparticle cell scaffold post-delivery through a 27G needle. Scale bar 10 μm. (B, D) Cell number after cellular incubation in basal media, or media supplemented with 0.25% CMC and 0.25% pluronic F127 after 1 and 24 h compared to an initial seeding density (B), (D) Cell number post simulated delivery through a needle free of 27G needle system using basal media or media supplemented with 0.25% CMC and 0.25% pluronic F127. Statistical analysis performed using ANOVA, all statistical tests show $p > 0.05^{*}$, $p > 0.01^{**}$, $p > 0.001^{***}$, $p > 0.0001^{****}$, bars represent mean of 3–6 repeats with SEM error bars.

2011; Xu et al., 1995). The addition of these molecules to the formulation may therefore impact the shear forces particles are exposed to during ejection. To explore the relative importance of particle delivery yield, surface wetting, and ejection shear in controlling drug delivery, further studies should be performed to independently isolate these variables.

3.5. Modified viscosity formulations for the delivery of microparticles-cell scaffolds

Next, we investigated the effect of our modified viscosity formulation on cell viability. At low concentrations, both CMC and pluronic acid moieties have been shown to protect cells from detrimental effects due to excessive shear forces (Gallardo Rodriguez et al., 2011; Xu et al., 1995). We examined the viability of human mesenchymal stem cells (MSCs) at 1 h and 24 h in media supplemented with our formulation. Fig. 3B shows cell proliferation calibrated using the Presto Blue metabolic assay in both conditions. In both solutions, cells demonstrate a similar viability at 1 h, and at 24 h show a significant increase in metabolic activity compared to 1 h. Cells remained viable in the formulation for up to 24 h, though there was a reduction in metabolic activity after incubation for 24 h in our formulation compared to basal media. At the 1 h timepoint, which represents a realistic timeframe for the clinical administration of cell-particle systems, there was no significant difference in viability between cells cultured in basal media and the 0.25% pluronic F127 0.25% CMC formulation, indicating this formulation may be suitable for the *in vivo* delivery of microparticle-cell systems.

Finally, we investigated the delivery of cells together with PLGA microparticles through syringes, either with or without a 27G needle, in basal media or our 0.25% CMC and 0.25% pluronic F127 formulation. Fig. 3C shows SEM images of cells delivered together with PLGA microparticle scaffolds, and demonstrates the integrity of both the particles and the cells post injection, and that the PLGA particles can be used to provide a scaffold for the cells. Fig. 3D illustrates that in both needle free and 27G needle delivery systems, the use of the 0.25% CMC and 0.25% pluronic F127 formulation significantly increased cell delivery by 200–400% compared to basal media. These results are comparable

to our earlier studies demonstrating enhanced delivery in microparticle only systems, and support our conclusion that basal media supplemented with 0.25% CMC and 0.25% pluronic F127 provides a formulation that can enhance delivery of PLGA microparticles, and microparticle-cell combination therapeutics, in needle delivery systems without compromising cell viability. We believe these results show the first needle delivery of a porous PLGA microparticle-MSC system, as proof-of-concept drug-eluting microparticle-cell scaffolds capable of combining a biodegradable cell support with localised drug delivery. Encapsulating soluble factors which direct host- or transplanted cell behaviour within these microparticle scaffolds would increase their versatility and make them a powerful tool for cell transplant.

4. Conclusion

The delivery of a controlled dose of microparticles is crucial for therapeutic applications. We find that the addition of viscosity modifiers can enhance particle delivery up to 520% across needle gauges between 21 and 30G, and identify a formulation of basal media supplemented with 0.25% pluronic F127 and 0.25% CMC as providing an optimal system. Although the polymers explored here increase delivery across all needle sizes tested, our results indicate that selection of an appropriate needle is also an important parameter to consider. We tested the biocompatibility of our lead formulation, finding that cells remain viable in the formulation for up to 24 h, and demonstrate that this formulation is suitable for the improved *in vitro* delivery of drug eluting PLGA microparticles, and microparticle-cell scaffolds. These microparticle-cell scaffolds offer the potential to simultaneously support cells for transplant and modulate the host environment/transplanted cell behaviour through the controlled release of pharmaceuticals. Together, these results pave the way for further exploration of microparticle-cell scaffold and delivery systems for *in vivo* cell transplantation. Additionally, these results have important implications for the application of microparticle and microparticle-cell therapeutics, and may also apply to other polymer based pharmaceuticals or protein biologics delivered by needle. In many cases, *in vitro* testing and clinical applications use different delivery strategies, with different needle and formulation systems, which could lead to differences in administered therapeutic dose. In order to match *in vitro*, pre-clinical and therapeutic outcomes, administration parameters (such as needle diameter and delivery formulation) should be carefully considered, and ideally conserved between pre-clinical and therapeutic applications.

Acknowledgements

We would like to acknowledge funding provided by the UKRMP Acellular Hub and the EPSRC E-TERM program, which helped to support this work.

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