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

3

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19 **Keywords:** high viscosity formulation; microparticle delivery; cell particle scaffolds; needle  
20 gauge

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22

## 23 **Abstract**

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

39

## 40 **1. Introduction**

41 Poly (DL-lactic acid-co-glycolic acid) (PLGA) materials are widely used therapeutics with  
42 applications in drug delivery,<sup>[1-3]</sup> tissue engineering,<sup>[4]</sup> and cellular scaffolding.<sup>[5, 6]</sup> In drug  
43 delivery applications, PLGA microparticles offer tunable, biodegradable kinetic release  
44 profiles. They are FDA approved for a variety of applications, and can often be administered  
45 *via* localised or systemic injection.<sup>[2, 7, 8]</sup> Larger 3-dimensional PLGA structures have also  
46 been surgically implanted as cellular scaffolds for regenerative medicine,<sup>[9, 10]</sup> however there  
47 are limited examples of systems combining the extracellular support matrix provided by

48 PLGA <sup>[5, 11]</sup> with the tunable kinetic release of soluble factors.<sup>[7]</sup> Through this combination of  
49 a physical support matrix and soluble cellular cues, microparticle cell scaffold systems with  
50 controlled release properties are able to support and direct transplanted cell behaviour.

51 A current challenge in the delivery of microparticles, cells, or microparticle-cell combination  
52 therapies lies in maintaining an effective therapeutic dose across varied application routes. In  
53 many systems, a common clinical administration route is the localised injection of materials  
54 using either pre-filled or self-filled syringes together with a needle.<sup>[12-15]</sup> Selection of an  
55 appropriate needle gauge depends on the therapeutic application; finer needles of 29G are  
56 often used for spatially accurate delivery of materials to the spinal cord, compared to 14G  
57 needles frequently used for intramuscular injection.<sup>[16-20]</sup> In cell-only systems, recent studies  
58 have suggested that injection parameters (including needle gauge, flow rate and applied force)  
59 can affect both the number of cells delivered and the ability of these cells to undergo  
60 phenotypic differentiation,<sup>[21-24]</sup> however these studies have yet to be applied to delivery of  
61 acellular microparticle systems.

62 Maintaining an effective therapeutic dose across a broad range of administration routes  
63 remains an ongoing consideration for the clinical application of drug delivery systems.<sup>[25] [26]</sup>  
64 To our knowledge, there has yet to be a study on the effect of injection parameters on delivery  
65 of either PLGA microparticles alone, or PLGA microparticles in conjunction with cells.  
66 Here, we explore modified viscosity systems to enhance both microparticle delivery and  
67 microparticle-cell scaffold delivery across a range of clinically relevant needle gauges. First,  
68 we explore PLGA particle delivery across a range of needle gauges with controlled plunger  
69 force. Next, we investigate modified delivery formulations using the thickening agent  
70 carboxymethylcellulose (CMC)<sup>[27]</sup> and the amphiphilic polymer pluronic F127,<sup>[28]</sup> probing  
71 their ability to modify viscosity and their effect on particle delivery across needle sizes.  
72 Finally, we investigate the effect of our lead formulation on the viability of human MSCs, and

73 demonstrate the use of our formulation in delivering multifunctional PLGA microparticle  
74 scaffolds with human MSCs *in vitro*.

## 75 **2. Materials and Methods**

### 76 *2.1 Fabrication of PLGA microparticles*

77 Non-porous PLGA particles were fabricated using 20% PLGA (50:50, 52 kDa Lakeshore  
78 Biomaterials) in dichloromethane (DCM) (Fischer) by either a single or double emulsion  
79 method. In the single emulsion method, the polymer solution was homogenised in 250 mL  
80 of 0.3% polyvinyl alcohol (13-24 kDa, Sigma-Aldrich) using a high speed Silverson L5M  
81 homogeniser. The resulting emulsion was left stirring at 300 RPM until particles hardened.  
82 In the double emulsion method, 100 µL of an aqueous solution containing 10 mg  
83 Amoxicillin (Abcam) was homogenised in the polymer solution. The resultant primary  
84 water in oil (w/o) emulsion was then homogenised again in the 0.3% PVA and the resultant  
85 water in oil in water (w/o/w) double emulsion was left stirring until particles hardened.  
86 Particles were extracted by centrifugation, washed, and lyophilised before being stored at -  
87 20°C until use. Porous PLGA particles were produced using a double emulsion method as  
88 previously described.<sup>[6]</sup> Briefly, 20% (w/v) PLGA in dichloromethane was treated with  
89 phosphate buffered saline (PBS, Gibco) as a porogen. Post fabrication, the particles were  
90 treated with ethanolic sodium hydroxide (sodium hydroxide (Sigma-Aldrich) and absolute  
91 ethanol (Fischer)) to enhance surface porosity. The particles were then extracted by  
92 centrifugation, washed, and lyophilised before being stored at -20°C until use.

93 Particles were characterised using scanning electron microscopy and laser diffraction.  
94 Briefly, particles were loaded onto carbon disks on aluminium stubs (Agar Scientific),  
95 sputter coated with gold (Balzers Union Ltd.) and imaged on an JEOL 6060L system. The  
96 mean diameter and particle size distribution were analysed using a Coulter LS230 particle

97 size analyser (Beckman, UK). Particle size distribution was then determined as a function  
98 of the particle diffraction and plotted as a function of volume percentage.

### 99 *2.2 Delivery formulations*

100 Particles were resuspended at 5 mg/mL in DMEM (Gibco), containing between 0-10%  
101 pluronic F127 (Sigma-Aldrich) or 0-10% medium viscosity sodium carboxymethylcellulose  
102 (CMC) (Sigma-Aldrich). Combined formulations containing between 0-0.5% pluronic and 0-  
103 0.5% CMC were also prepared. Formulation solutions were kept at 4°C until use. Solution  
104 viscosity was measured using a rheometer with cone and plate geometry at 0.1° angle (Anton  
105 Parr- Physica MCR 301)), using a shear ramp from 0-100 1/s at 25°C.

### 106 *2.3 Particle injection*

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

### 119 *2.4 Injection forces and calculated shear rates*

120 For each needle-syringe combination, the initial and glide force were determined using a  
121 texture analyser (TA.HD *plus*, Stable micro systems). 1 mL of formulation was loaded into a  
122 1 mL syringe (BD), and fitted with an appropriate needle into the injection rig. A 10 mm  
123 cylinder probe was lowered into contact with the plunger, with no pre-test force, before a 1  
124 mm/s ejection rate was applied in compression mode. The initial force was calculated as the  
125 force required to overcome the resistance to movement of the plunger, whereas the glide force  
126 was calculated as the average force required to evacuate the syringe at 1 mm/s. For  
127 formulations tested with microparticles, a concentration of 5 mg/mL particles suspended in 1  
128 mL solution was used. Shear rates were calculated using Poiseuilles equation;

129 
$$\gamma = \frac{4Q}{\pi r^3}$$

130 Where  $\gamma$  is shear rate in  $s^{-1}$ , Q is flow rate in  $cm^3/s$ , and r is needle radius in cm. Shear rates  
131 were calculated using both experimental flow rate (for 1 mm/s plunger ejection) and  
132 theoretical flow rates 1 ml/hour and 20 ml/hour expected to be used in clinic, described in  
133 Table 2.

#### 134 *2.5 PLGA microparticle release studies*

135 *In vitro* testing of the controlled release of Amoxicillin encapsulated within PLGA  
136 microparticles was performed using Transwell inserts (Corning, UK). 25 mg of PLGA  
137 microparticles were suspended in 1.5 mL of the described formulations, and incubated at  
138 37 °C. The concentration of Amoxicillin in release medium was quantified by UV  
139 detection at 300 nm using a plate reader (Tecan) with concentration determined from a  
140 calibration curve.

#### 141 *2.6 Cell viability*

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

### 149 *2.7 Injection of cells cultured on particles*

150 Porous particles were treated with Tween and then antibiotic/antimycotic solution (Sigma-  
151 Aldrich). Commercially available human mesenchymal stem cells (MSCs) (Japanese Stem  
152 Cell Bank) were seeded at 200,000 per well in 12-well plates, with 8mg PLGA particles  
153 added per well, and incubated overnight at 37 °C in DMEM medium supplemented with 10%  
154 foetal calf serum, 1% antibiotic/ antimycotic solution, 1% L-glutamine (2 mM) and 1% non-  
155 essential amino acids (Sigma-Aldrich). Wells were centrifuged, and the cell pellet re-  
156 suspended in DMEM or formulation conditions. As described previously, this suspension was  
157 injected into a fresh 12-well plate, and incubated for 10 minutes with 10% Presto blue at  
158 37 °C. Cell number per well was quantified using a Tecan plate reader. Samples were  
159 formalin fixed for 20 minutes at room temperature, washed with PBS multiple times and then  
160 imaged by SEM.

### 161 *2.8 Delivery efficacy*

162 Delivery efficacy was calculated by comparing the number of particles delivered using a  
163 specific formulation and needle combination to the number of particles delivered in a basal  
164 media solution. Particles were counted using a hemocytometer. For example, to compare the  
165 delivery efficacy of particles suspended in basal media through needle-free syringes and 27G

166 needles, a suspension of PLGA particles was loaded into at least six identical syringes, three  
167 of which were uncapped and three capped with 27G needles. Syringes were loaded onto the  
168 controlled rate syringe pump, and ejected at constant plunger speed of 1 mm/s. An aliquot of  
169 the ejected solution was transferred to a hemocytometer and the number of ejected particles  
170 counted. To calculate delivery efficiency, we averaged the number of particles for each  
171 condition and calculated efficacy as follows;

$$172 \quad \text{delivery efficacy} = \frac{\text{average number of particles delivered in condition X}}{\text{average number of particles delivered in basal media}} \times 100$$

## 173 2.9 Statistical analysis

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

## 181 3. Results and Discussion

### 182 3.1 The effect of needle gauge on microparticle delivery

183 We fabricated PLGA microparticles of 27  $\mu\text{m}$  diameter as described in section 2.1 (**Figure**  
184 **1A**), and investigated delivery efficacy through needles between 21-30G, corresponding to  
185 internal needle diameters currently used in clinic ranging from over 500  $\mu\text{m}$  to around 160  $\mu\text{m}$   
186 (**Table 1**). Using a syringe pump set up with constant flow rate of 1 mm/s, we evaluated the  
187 ejection of PLGA microparticles suspended in basal media solution through a range of needle  
188 gauges (**Figure 1B**). All needle gauges tested were able to deliver the microparticles, however

189 we find that narrower needle gauges of 27G and 30G failed to deliver microparticle solutions  
190 as effectively as either needle free or large-bore needle systems, with delivery efficacy  
191 reduced to 61% in 27G needles compared to needle free systems. Given the internal needle  
192 diameters of 27G and 30G needles (210  $\mu\text{m}$  and 160  $\mu\text{m}$  respectively) are wider than the 26.9  
193  $\mu\text{m}$  (+/- SD 11.2  $\mu\text{m}$ ) diameter of the microparticles (**Figure 1A**), this suggests that the  
194 particle delivery through narrow gauge needles is affected not only by particle size, but also  
195 the dynamics of the fluid ejection from the syringe. To explore this further, we investigated  
196 the effect of altering the solution viscosity, and so ejection fluid dynamics, of the delivery  
197 formulation in needle delivery systems.

### 198 **3.2 PLGA microparticle delivery using modified formulations**

199 Formulations containing the thickening agent carboxymethylcellulose (CMC) (**Figure 1C**),  
200 pluronic F127 (**Figure 1D**) to modulate wettability, and CMC/pluronic F127 (**Figure 1E**)  
201 combination formulations were tested for their ability to deliver PLGA microparticles. Figure  
202 1 demonstrates that increasing concentrations of either CMC or pluronic F127 in media  
203 significantly increased particle delivery, illustrated by the 400% increase of particle delivery  
204 in systems using either 5% pluronic F127 or 2% CMC, compared to un-supplemented media.  
205 We then investigated combination formulations with compositions of between 0.125-0.5% of  
206 CMC and pluronic F127 in media. Figure 1E illustrates that particle delivery was significantly  
207 improved in all combination formulations tested, and broadly increased as the concentration  
208 of CMC/pluronic F127 in solution increased.

209 We calculated the shear rate used during our experimental injections at a constant plunger rate  
210 of 1 mm/s (**Table 2**). These values suggest our experimental injection system experiences low  
211 shear between 150-500/s. We examined the effect of formulation composition on viscosity at  
212 comparable shear rates between 1-100/s. Comparing the formulations, Figure 1F illustrates  
213 that the viscosity of each composition remains broadly constant against increasing shear

214 between 1-100/s. In systems with constant CMC concentration and varying pluronic F127  
215 concentration, viscosity measurements are similar, whilst viscosity measurements roughly  
216 double as the concentration of CMC doubles in the formulation, suggesting that viscosity  
217 properties are broadly driven by the CMC concentration in solution.<sup>[29, 30]</sup> Given that many  
218 clinically administered injections are delivered at higher flow rates than those tested  
219 experimentally,<sup>[31, 32]</sup> we next calculated the expected flow rate and shear rate during more  
220 rapid administration through various needle gauges (Table 2). Needles injecting flow rates of  
221 1 ml/hour and 20 ml/hour (flow rates more commonly used for clinical infusion regimes)  
222 experience much higher shear rates through similar needle gauges.

223 At the shear rates tested here, supplementing basal media with pluronic acid and CMC can  
224 increase microparticle delivery. There are several potential explanations for this, including the  
225 changes in solution viscosity and the increased wetting of microparticles in these  
226 formulations. The increased solution viscosity may help to form a stable microparticle  
227 suspension during syringe evacuation, which prevents particles being forced to the side of the  
228 syringe. Particles at the side of syringe can be statically attracted to the syringe casing and are  
229 less likely to be ejected.<sup>[33]</sup> Additionally, Figure 1E indicates that increasing the total polymer  
230 concentration to above 0.5%, can increase delivery payload. It is likely that the negatively  
231 charged polymers help to combat static and attractive charges between the particles and the  
232 syringe, facilitating particle ejection.<sup>[8, 29]</sup> The combined effects of increased polymer  
233 concentration, particle wetting and increased viscosity, are likely responsible for the increase  
234 in delivery.

### 235 **3.3 Modified delivery formulations using clinical needle gauges**

236 All combination formulations tested demonstrated enhanced microparticle delivery compared  
237 to a basal media control in a 23G needle system. We then explored whether these combination  
238 formulations were able to enhance microparticle delivery across a range of clinically relevant

239 needle gauges.<sup>[16-18, 20, 34, 35]</sup> Figure 2A compares the number of microparticles delivered for  
240 each formulation in 21-27G needles, and Figure 2B summarises the average delivery across  
241 all needle gauges tested. There is a significant increase in particle delivery across all needle  
242 gauges using CMC and pluronic F127 formulations in comparison to basal media ( $p < 0.001$  in  
243 all formulations, and all needle gauges, Turkey's multiple comparison tests and two-way  
244 ANOVA), with delivery increasing on average between 300-400% compared to particles  
245 delivered in basal media in the same needle gauge.

246 We further explored needle blockage and injection failure for each of these formulations  
247 (**Figure 2C**) in needle gauges. Figure 2C demonstrates that the 0.25% pluronic F127 and  
248 0.25% CMC formulation had no injection failures compared to other formulations which  
249 occasionally resulted in a blocked needle injection failure. Considering this 0.25% pluronic  
250 F127 and 0.25% CMC formulation in more detail, we find that microparticle delivery is  
251 significantly increased in all needle gauges tested using this formulation compared to a basal  
252 media control (**Figure 2D**), with increases in delivery between 320-750% compared to basal  
253 media (**Table 3**), and an average increase in microparticle delivery of 520% across all needle  
254 gauges. This formulation is therefore broadly applicable to a range of clinical needle gauges.

### 255 **3.4 Modified viscosity formulations for the delivery of drug loaded microparticles**

256 We investigated the applicability of the lead 0.25% CMC 0.25% pluronic F127 formulation  
257 for the delivery of drug-eluting microparticles and microparticle-cell scaffold therapeutics.  
258 First, the release profile of amoxicillin from amoxicillin loaded PLGA microparticles was  
259 tested, exploring microparticles delivered through a 27G needle with and without the  
260 modified viscosity formulation (**Figure 3A**). As expected, the total amount of amoxicillin  
261 released was increased when our delivery formulation was used. As the kinetics of release  
262 remained broadly unaffected, we suggest that this is due to the increased delivery yield using  
263 the 0.25% CMC 0.25% pluronic F127 formulation.

264 Interestingly, the addition of pluronic acid and CMC may also alter microparticle surface  
265 wetting and shear forces experienced during ejection, which could also influence drug release  
266 kinetics. The hydrophilicity of PLGA surfaces affects polymer degradation kinetics, and is  
267 correlated with both co-polymer composition and liquid solvent polarity.<sup>[36, 37]</sup> The addition of  
268 CMC and pluronic acid to the basal media formulation may alter the polarity of the liquid  
269 phase, and could directly impact microparticle surface wetting and so drug release kinetics.  
270 Formulations containing CMC and pluronic acid have also been shown to alter shear forces  
271 experienced by cells during stirred culture.<sup>[38, 39]</sup> The addition of these molecules to the  
272 formulation may therefore impact the shear forces particles are exposed to during ejection. To  
273 explore the relative importance of particle delivery yield, surface wetting, and ejection shear  
274 in controlling drug delivery, further studies should be performed to independently isolate  
275 these variables.

### 276 **3.5 Modified viscosity formulations for the delivery of microparticles-cell scaffolds**

277 Next, we investigated the effect of our modified viscosity formulation on cell viability. At low  
278 concentrations, both CMC and pluronic acid moieties have been shown to protect cells from  
279 detrimental effects due to excessive shear forces.<sup>[38, 39]</sup> We examined the viability of human  
280 mesenchymal stem cells (MSCs) at 1 hour and 24 hours in media supplemented with our  
281 formulation. Figure 3B shows cell proliferation calibrated using the Presto Blue metabolic  
282 assay in both conditions. In both solutions, cells demonstrate a similar viability at 1 hour, and  
283 at 24 hours show a significant increase in metabolic activity compared to 1 hour. Cells  
284 remained viable in the formulation for up to 24 hours, though there was a reduction in  
285 metabolic activity after incubation for 24 hours in our formulation compared to basal media.  
286 At the 1 hour timepoint, which represents a realistic timeframe for the clinical administration  
287 of cell-particle systems, there was no significant difference in viability between cells cultured

288 in basal media and the 0.25% pluronic F127 0.25% CMC formulation, indicating this  
289 formulation may be suitable for the *in vivo* delivery of microparticle-cell systems.

290 Finally, we investigated the delivery of cells together with PLGA microparticles through  
291 syringes, either with or without a 27G needle, in basal media or our 0.25% CMC and 0.25%  
292 pluronic F127 formulation. Figure 3C shows SEM images of cells delivered together with  
293 PLGA microparticle scaffolds, and demonstrates the integrity of both the particles and the  
294 cells post injection, and that the PLGA particles can be used to provide a scaffold for the cells.  
295 Figure 3D illustrates that in both needle free and 27G needle delivery systems, the use of the  
296 0.25% CMC and 0.25% pluronic F127 formulation significantly increased cell delivery by  
297 200-400% compared to basal media. These results are comparable to our earlier studies  
298 demonstrating enhanced delivery in microparticle only systems, and support our conclusion  
299 that basal media supplemented with 0.25% CMC and 0.25% pluronic F127 provides a  
300 formulation that can enhance delivery of PLGA microparticles, and microparticle-cell  
301 combination therapeutics, in needle delivery systems without compromising cell viability. We  
302 believe these results show the first needle delivery of a porous PLGA microparticle-MSC  
303 system, as proof-of-concept drug-eluting microparticle-cell scaffolds capable of combining a  
304 biodegradable cell support with localised drug delivery. Encapsulating soluble factors which  
305 direct host- or transplanted cell behaviour within these microparticle scaffolds would increase  
306 their versatility and make them a powerful tool for cell transplant.

#### 307 **4. Conclusion**

308 The delivery of a controlled dose of microparticles is crucial for therapeutic applications. We  
309 find that the addition of viscosity modifiers can enhance particle delivery up to 520% across  
310 needle gauges between 21-30G, and identify a formulation of basal media supplemented with  
311 0.25% pluronic F127 and 0.25% CMC as providing an optimal system. Although the  
312 polymers explored here increase delivery across all needle sizes tested, our results indicate

313 that selection of an appropriate needle is also an important parameter to consider. We tested  
314 the biocompatibility of our lead formulation, finding that cells remain viable in the  
315 formulation for up to 24 hours, and demonstrate that this formulation is suitable for the  
316 improved *in vitro* delivery of drug eluting PLGA microparticles, and microparticle-cell  
317 scaffolds. These microparticle-cell scaffolds offer the potential to simultaneously support cells  
318 for transplant and modulate the host environment/transplanted cell behaviour through the  
319 controlled release of pharmaceuticals. Together, these results pave the way for further  
320 exploration of microparticle-cell scaffold and delivery systems for *in vivo* cell transplantation.  
321 Additionally, these results have important implications for the application of microparticle  
322 and microparticle-cell therapeutics, and may also apply to other polymer based  
323 pharmaceuticals or protein biologics delivered by needle. In many cases, *in vitro* testing and  
324 clinical applications use different delivery strategies, with different needle and formulation  
325 systems, which could lead to differences in administered therapeutic dose. In order to match  
326 *in vitro*, pre-clinical and therapeutic outcomes, administration parameters (such as needle  
327 diameter and delivery formulation) should be carefully considered, and ideally conserved  
328 between pre-clinical and therapeutic applications.

329

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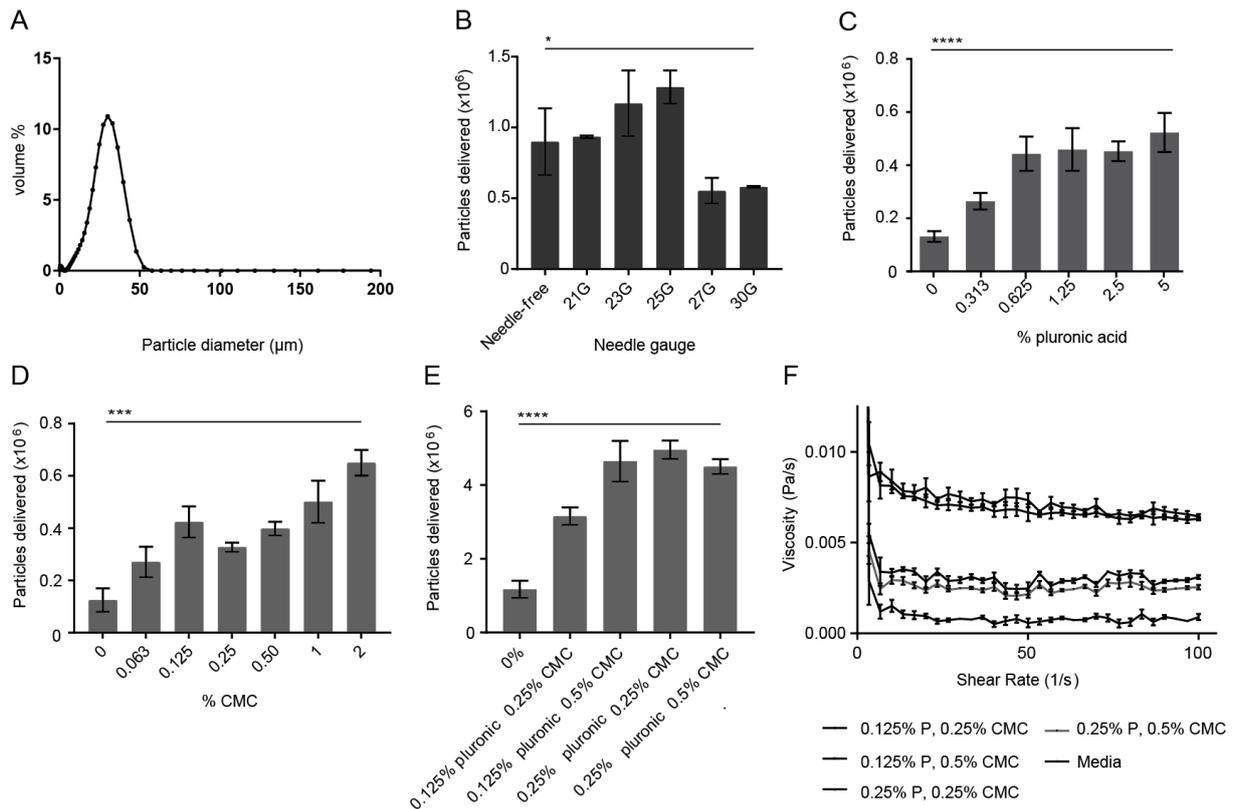
336 **References**

- 337 1. Rao, D.A., et al., *Biodegradable PLGA based nanoparticles for sustained regional*  
338 *lymphatic drug delivery*. J Pharm Sci, 2010. **99**(4): p. 2018-31.
- 339 2. Rafati, A., et al., *Chemical and spatial analysis of protein loaded PLGA microspheres*  
340 *for drug delivery applications*. Journal of Controlled Release, 2012. **162**(2): p. 321-  
341 329.
- 342 3. Simon-Yarza, T., et al., *PEGylated-PLGA microparticles containing VEGF for long*  
343 *term drug delivery*. International Journal of Pharmaceutics, 2013. **440**(1): p. 13-18.
- 344 4. Park, J.S., et al., *Stem cell differentiation-related protein-loaded PLGA microspheres*  
345 *as a novel platform micro-typed scaffold for chondrogenesis*. Biomedical Materials,  
346 2016. **11**(5).
- 347 5. Lee, Y.S., et al., *Development of porous PLGA/PEI1.8k biodegradable microspheres*  
348 *for the delivery of mesenchymal stem cells (MSCs)*. Journal of Controlled Release,  
349 2015. **205**: p. 128-133.
- 350 6. Qutachi, O., et al., *Injectable and porous PLGA microspheres that form highly porous*  
351 *scaffolds at body temperature*. Acta Biomater, 2014. **10**(12): p. 5090-8.
- 352 7. Cappellano, G., et al., *Subcutaneous inverse vaccination with PLGA particles loaded*  
353 *with a MOG peptide and IL-10 decreases the severity of experimental autoimmune*  
354 *encephalomyelitis*. Vaccine, 2014. **32**(43): p. 5681-5689.
- 355 8. Park, C.H., et al., *Needle-free transdermal delivery using PLGA nanoparticles: Effect*  
356 *of particle size, injection pressure and syringe orifice diameter*. Colloids and Surfaces  
357 B-Biointerfaces, 2014. **123**: p. 710-715.
- 358 9. Hernandez, R.M., et al., *Microcapsules and microcarriers for in situ cell delivery*.  
359 *Advanced Drug Delivery Reviews*, 2010. **62**(7-8): p. 711-730.
- 360 10. Kochenderfer, J.N., et al., *A Phase I Clinical Trial of Treatment of B Cell*  
361 *Malignancies with Autologous Anti CD19 CAR Transduced T Cells*. Blood, 2010.  
362 **116**(21): p. 1179-1180.
- 363 11. Han, K.-S., et al., *Effect of demineralized bone particle/poly(lactic-co-glycolic acid)*  
364 *scaffolds on the attachment and proliferation of mesenchymal stem cells*. Journal of  
365 *Biomaterials Science-Polymer Edition*, 2015. **26**(2): p. 92-110.
- 366 12. Garbayo, E., et al., *Catheter-based Intramyocardial Injection of FGF1 or NRG1-*  
367 *loaded MPs Improves Cardiac Function in a Preclinical Model of Ischemia-*  
368 *Reperfusion*. Scientific Reports, 2016. **6**.
- 369 13. McHugh, K.J., et al., *Single-injection vaccines: Progress, challenges, and*  
370 *opportunities*. Journal of Controlled Release, 2015. **219**: p. 596-609.
- 371 14. Fu, J., et al., *Subconjunctival Delivery of Dorzolamide-Loaded Poly(ether-anhydride)*  
372 *Microparticles Produces Sustained Lowering of Intraocular Pressure in Rabbits*.  
373 *Molecular Pharmaceutics*, 2016. **13**(9): p. 2987-2995.
- 374 15. Kim, Y.-C., et al., *Transplantation of Mesenchymal Stem Cells for Acute Spinal Cord*  
375 *Injury in Rats: Comparative Study between Intralesional Injection and Scaffold Based*  
376 *Transplantation*. Journal of Korean Medical Science, 2016. **31**(9): p. 1373-1382.
- 377 16. Dittmann, M., et al., *9 YEARS OF CLINICAL-EXPERIENCE WITH 29 GAUGE*  
378 *SPINAL NEEDLES*. British Journal of Anaesthesia, 1993. **70**: p. 65-65.
- 379 17. Mavrogenis, G., et al., *25-gauge histology needle versus 22-gauge cytology needle in*  
380 *endoscopic ultrasonography-guided sampling of pancreatic lesions and*  
381 *lymphadenopathy*. Endoscopy International Open, 2015. **3**(1): p. E63-E68.
- 382 18. Songur, N., et al., *Comparison of 19-and 22-gauge needles in EUS-guided fine needle*  
383 *aspiration in patients with mediastinal masses and lymph nodes*. Turkish Journal of  
384 *Gastroenterology*, 2011. **22**(5): p. 472-478.

- 385 19. Sivera, F., R. Aragon, and E. Pascual, *First metatarsophalangeal joint aspiration*  
386 *using a 29-gauge needle*. Annals of the Rheumatic Diseases, 2008. **67**(2): p. 273-275.
- 387 20. Raftesath, D. and M. Fitzgerald, *Influence of needle gauge used for venipuncture on*  
388 *automated platelet count and coagulation profile in dogs*. Australian Veterinary  
389 Journal, 2014. **92**(3): p. N16-N16.
- 390 21. Mamidi, M.K., et al., *Impact of passing mesenchymal stem cells through smaller bore*  
391 *size needles for subsequent use in patients for clinical or cosmetic indications*. J  
392 Transl Med, 2012. **10**: p. 229.
- 393 22. Amer, M.H., et al., *A Detailed Assessment of Varying Ejection Rate on Delivery*  
394 *Efficiency of Mesenchymal Stem Cells Using Narrow-Bore Needles*. Stem Cells  
395 Translational Medicine, 2016. **5**(3): p. 366-378.
- 396 23. Amer, M.H., L.J. White, and K.M. Shakesheff, *The effect of injection using narrow-*  
397 *bore needles on mammalian cells: administration and formulation considerations for*  
398 *cell therapies*. Journal of Pharmacy and Pharmacology, 2015. **67**(5): p. 640-650.
- 399 24. Amer, M.H., et al., *Evaluation of Delivery of Mesenchymal Stem Cells using Small-*  
400 *Gauge Needles: Tailoring Administration of Cell-Based Therapies for Efficient*  
401 *Clinical Translation*. Tissue Engineering Part A, 2015. **21**: p. S265-S265.
- 402 25. Cilurzo, F., et al., *Injectability evaluation: an open issue*. AAPS PharmSciTech, 2011.  
403 **12**(2): p. 604-9.
- 404 26. Kearney, C.J. and D.J. Mooney, *Macroscale delivery systems for molecular and*  
405 *cellular payloads*. Nature Materials, 2013. **12**(11): p. 1004-1017.
- 406 27. Nakanishi, Y., [*Studies on pharmaceutical suspensions. 3. Viscosities of the dispersed*  
407 *systems of barium sulfate in MC and CMC aqueous solutions*]. Yakugaku Zasshi,  
408 1966. **86**(11): p. 997-1000.
- 409 28. Prameela, G.K., et al., *Physicochemical perspectives (aggregation, structure and*  
410 *dynamics) of interaction between pluronic (L31) and surfactant (SDS)*. Phys Chem  
411 Chem Phys, 2015. **17**(45): p. 30560-9.
- 412 29. Voigt, M., M. Koerber, and R. Bodmeier, *Improved physical stability and injectability*  
413 *of non-aqueous in situ PLGA microparticle forming emulsions*. International Journal  
414 of Pharmaceutics, 2012. **434**(1-2): p. 251-256.
- 415 30. Ahmed, T.A., et al., *In vitro release, rheological, and stability studies of mefenamic*  
416 *acid coprecipitates in topical formulations*. Pharmaceutical Development and  
417 Technology, 2011. **16**(5): p. 497-510.
- 418 31. Nguyen, N.T., X.Y. Huang, and T.K. Chuan, *MEMS-micropumps: A review*. Journal  
419 of Fluids Engineering-Transactions of the Asme, 2002. **124**(2): p. 384-392.
- 420 32. Salman, D., et al., *Evaluation of the performance of elastomeric pumps in practice:*  
421 *are we under-delivering on chemotherapy treatments?* Current Medical Research and  
422 Opinion, 2017. **33**(12): p. 2153-2159.
- 423 33. Whitaker, M.A., et al., *Particle size and shape effects in medical syringe needles:*  
424 *experiments and simulations for polymer microparticle injection*. Journal of Materials  
425 Science-Materials in Medicine, 2011. **22**(8): p. 1975-1983.
- 426 34. Gill, H.S. and M.R. Prausnitz, *Does needle size matter?* J Diabetes Sci Technol, 2007.  
427 **1**(5): p. 725-9.
- 428 35. Fateh, M., et al., *Syringe-type and Needle Gauge Have No Role in Adverse Events*  
429 *Following DTwP Immunization: A Randomized Multicenter Trial*. Pediatric Infectious  
430 Disease Journal, 2014. **33**(9): p. E239-E246.
- 431 36. Chen, W.L., et al., *Effect of Particle Size on Drug Loading and Release Kinetics of*  
432 *Gefitinib-Loaded PLGA Microspheres*. Molecular Pharmaceutics, 2017. **14**(2): p. 459-  
433 467.
- 434 37. Vargha-Butler, E.I., et al., *Wettability of biodegradable surfaces*. Colloid and Polymer  
435 Science, 2001. **279**(12): p. 1160-1168.

- 436 38. Gallardo Rodriguez, J.J., et al., *Carboxymethyl cellulose and Pluronic F68 protect the*  
437 *dinoflagellate Protoceratium reticulatum against shear-associated damage.*  
438 *Bioprocess and biosystems engineering*, 2011. **34**(1): p. 3-12.
- 439 39. Xu, D., et al., *Studies of protective properties of pluronic and other agents on the*  
440 *hybridoma cell culture.* *Chinese journal of biotechnology*, 1995. **11**(2): p. 101-7.
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443 **Figure 1: Properties of pluronic F127 and CMC modified solutions** (A) Size distribution  
 444 of PLGA microparticles used in this study, measured by laser diffraction. (B) PLGA  
 445 microparticle delivery from a stock of 5mg/ml through syringes fitted with various needle  
 446 gauges (C, D, E) Increasing concentrations of pluronic F127 (C) CMC (D) and pluronic  
 447 F127/CMC combination formulations (E) were tested for their ability to deliver microparticles  
 448 from a stock solution through 27G needles (F) Viscosity measurements at increasing shear  
 449 rate, measured for combination formulations at 25°C. All statistical tests show one way  
 450 ANOVA where  $p > 0.05$  \*,  $p > 0.01$  \*\*,  $p > 0.001$  \*\*\*,  $p > 0.0001$  \*\*\*\*, bars represent mean of 3-  
 451 6 repeats with SEM error bars.

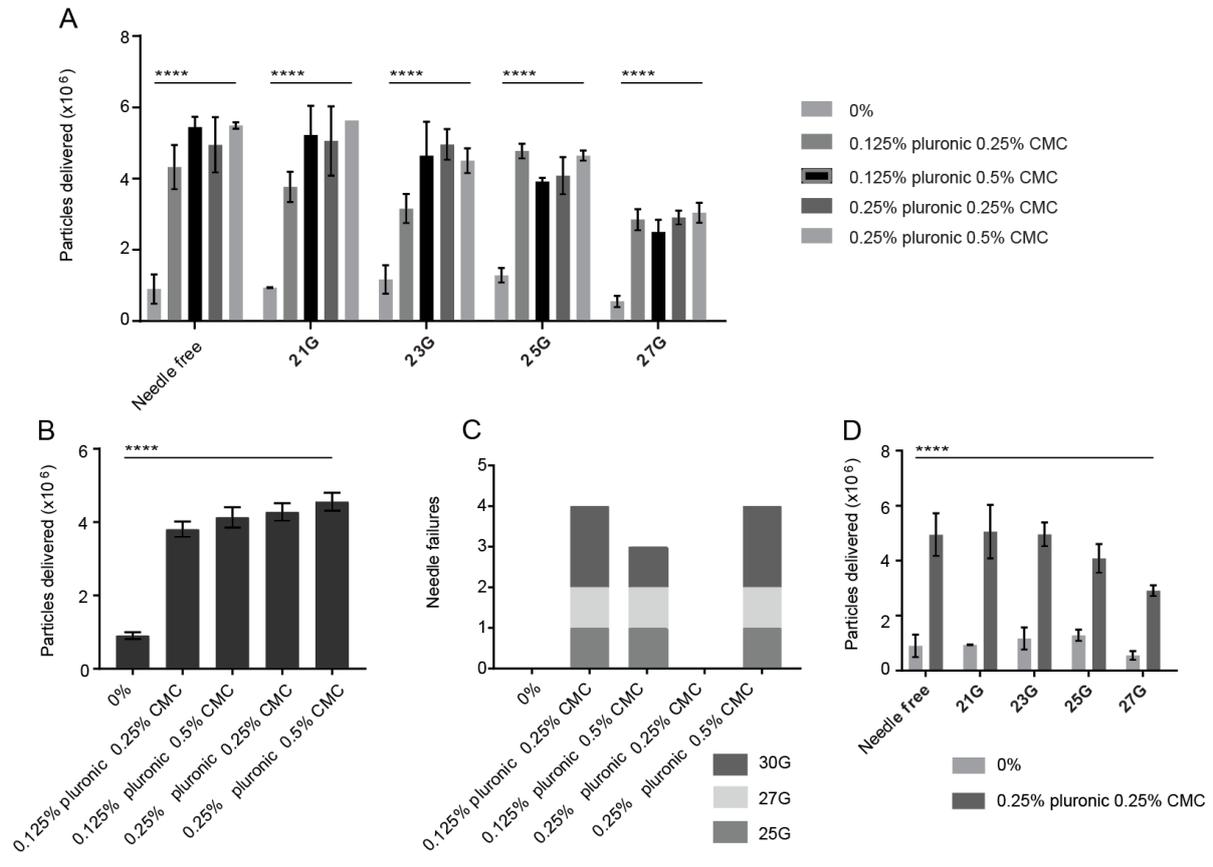


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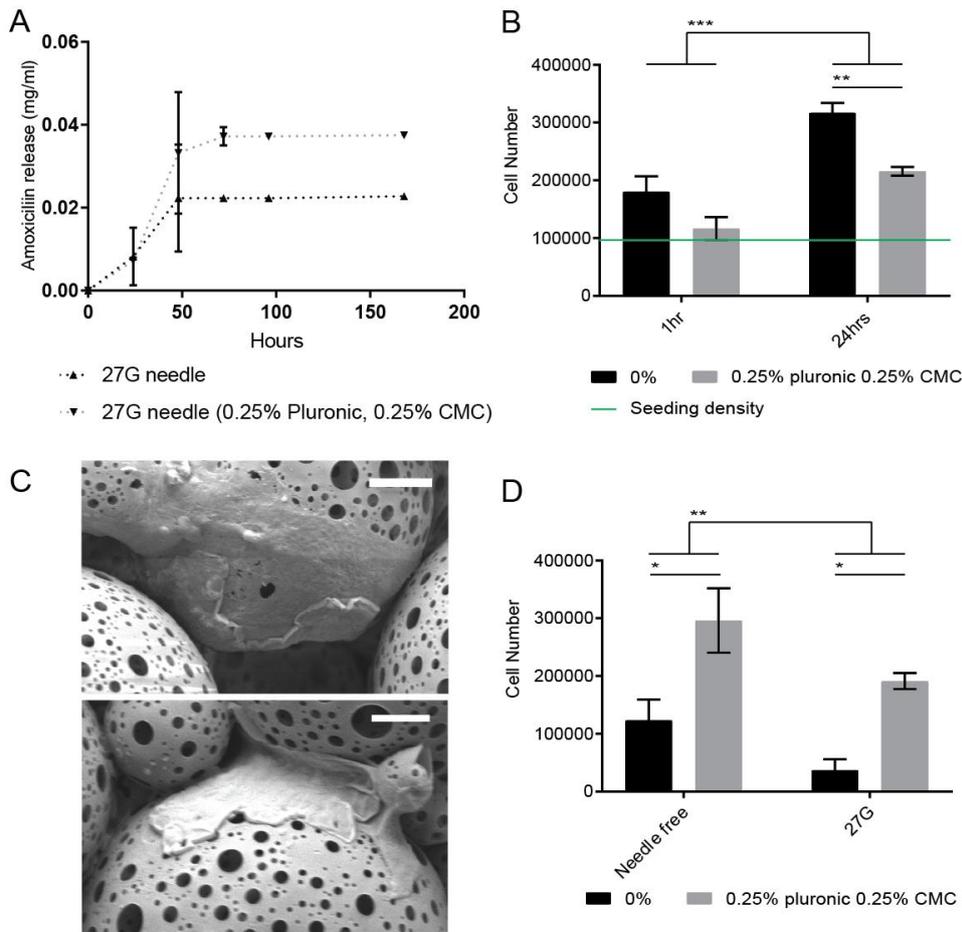
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455 **Figure 2: Modified solutions across needle gauges.** Increasing concentrations of (A)  
 456 pluronic F127/CMC combination formulations were tested for their ability to deliver  
 457 microparticles from a stock solution across a range of needle gauges. (B) Average delivery of  
 458 microparticles across all needle gauges (21-27G) in different combination formulations. (C)  
 459 Number of needle blockages in each formulation, n=10 for each formulation. Needles 30G,  
 460 27G, 25G, 23G and 21G were tested, with blockages found in 25-30G. (D) A comparison  
 461 between basal media and 0.25% pluronic 0.25% CMC for the delivery of microparticles  
 462 across needle gauges 21G-27G. All statistical tests show two way ANOVA where  $p > 0.05$  \*,  
 463  $p > 0.01$  \*\*,  $p > 0.001$  \*\*\*,  $p > 0.0001$  \*\*\*\*, bars represent mean of 3-6 repeats with SEM error  
 464 bars.



465

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



477

478

479 **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

480 **Table 2: Calculated shear rate through clinical needle gauges at varying flow** Shear rate  
 481 was calculated using Poiseuilles equation for needle gauges between 21-30G, using  
 482 experimental flow rates tested using 1 mm/s plunger speed, and theoretical flow rates of 1 or  
 483 20 ml/hour, all values rounded to 3 significant figures.

Needle gauge	Needle diameter (cm)	Experimental flow (plunger at 1mm/s)		Theoretical flow (1 ml/hour)	Theoretical flow (20 ml/hour)
		Q (cm <sup>3</sup> /s)	Shear rate (s <sup>-1</sup> )	Shear rate (s <sup>-1</sup> )	Shear rate (s <sup>-1</sup> )
30G	0.159	1.98E-07	503	704000	14100000
27G	0.210	3.46E-07	380	306000	6110000
25G	0.260	5.31E-07	308	161000	3220000
23G	0.337	8.92E-07	237	74000	1480000
21G	0.514	2.07E-06	156	20800	417000

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

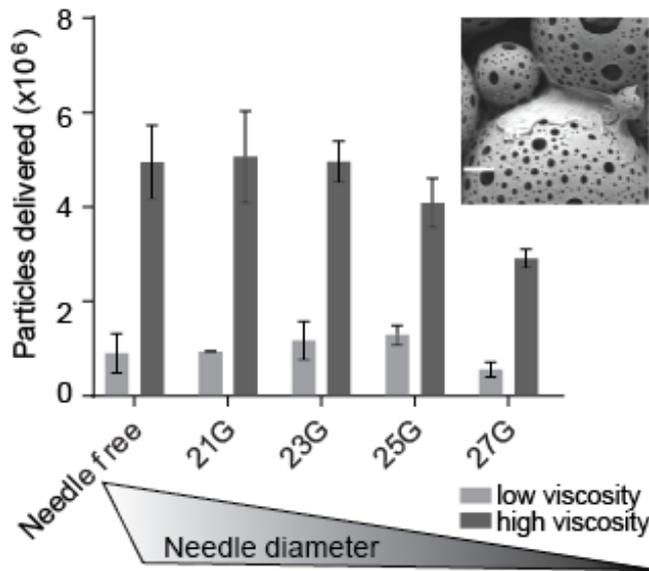
Needle system and media composition	Average number of particles delivered/ mL	Compare to: Needle system and media composition	Average number of particles delivered/ mL	Calculated delivery efficacy (%)
Needle free, basal media	0.90 x10 <sup>6</sup>	Needle free, 0.25% CMC 0.25% pluronic F127	4.95 x10 <sup>6</sup>	550%
21G, basal media	0.94 x10 <sup>6</sup>	21G, 0.25% CMC 0.25% pluronic F127	5.06 x10 <sup>6</sup>	538%
23G, basal media	1.17 x10 <sup>6</sup>	23G, 0.25% CMC 0.25% pluronic F127	4.96 x10 <sup>6</sup>	424%
25G, basal media	1.29 x10 <sup>6</sup>	25G, 0.25% CMC 0.25% pluronic F127	4.08 x10 <sup>6</sup>	316%
27G, basal media	0.55 x10 <sup>6</sup>	27G, 0.25% CMC 0.25% pluronic F127	2.91 x10 <sup>6</sup>	529%
30G, basal media	0.58 x10 <sup>6</sup>	30G, 0.25% CMC 0.25% pluronic F127	4.37 x10 <sup>6</sup>	753%

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

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497

498 **Graphical Entry:**



499

500 **Keywords: microparticle delivery, cell particle scaffolds, mesenchymal stem**  
501 **cell, needle gauge**

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