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Hydrogel Encapsulation to Improve Cell Viability during Syringe Needle Flow

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ABSTRACT: This work examines pluronic F-127 poloxamer for cell protection during injection through a syringe needle. Direct cell injection is a minimally invasive method for cell transplantation; however, it often results in poor cell viability. We proposed that encapsulating cells in this hydrogel would protect cells from detrimental mechanical forces during injection and increase cell viability. The hydrogel was tested at multiple weights and carbon nanobrush concentrations to determine how gel weight affects cell viability as well as to allow the gels to remain as electrically conductive scaffolds. This work assessed the ability of the hydrogel to prevent cell membrane bursting. We used D1 multipotent mouse bone marrow stromal precursor cells for this study. We found that the pressure drop increases with increasing weight of the gels. However, cell viability also increases as the weight of the gels increases. These results support the proposition that hydrogels can be used to protect cells during syringe needle injection. Since these hydrogels undergo a reverse phase transition, the gels can be used to transplant cells into the body in solution form through injection. The gels will then harden *in situ* to allow for cell proliferation and tissue regeneration at the desired site.

KEY WORDS: poloxamer hydrogel, reverse thermodynamic properties, cell viability, syringe needle flow, pressure drop, rheology, carbon nanobrushes

I. INTRODUCTION

In order for a material to be useful in bioengineering, it must have the following properties: biocompatibility, similar mechanical properties to the tissue it is replacing, promotion of tissue regeneration, sterilizability, viscosity, and ease of handling.[1] An area of growing interest in biomaterials research is using hydrogels for cell encapsulation.[2–8] These hydrogels are not only easy to handle, but they form a highly hydrated environment capable of supporting cell and tissue growth.[2] Another feature of hydrogels that makes them so prevalent in tissue engineering is their structural similarity to the native extracellular matrix of many tissues.[3] In addition, hydrogels can be processed with relative ease under mild conditions and, in many cases, are able to be delivered in a minimally invasive manner.[3] Due to these properties, hydrogels provide three-dimensional scaffolds with an environment very similar to that found *in vivo*, making them good candidates for encapsulating cells.[4]

A problem with many hydrogels intended for cell encapsulation, however, is that they require substantial environmental changes, such as pH, temperature, or ionic strength, to initiate the formation of the gel phase.[5] To avoid this, gels with the ability to undergo a reverse phase change are ideal. One such hydrogel, pluronic F-127 poloxamer, is a reverse phase change triblock copolymer mixture of polyethylene oxide and polypropylene oxide (PEO_{101}-PPO_{56}-PEO_{101}) that is hydrophilic and nonionic.[9] As such, it is a solution when kept

at cooler temperatures and solidifies into a gel as its temperature increases at its lower sol-gel boundary. With this in mind, it is possible to suspend cells in the hydrogel in solution form and then inject the solution through a syringe into the desired part of the body, where it will solidify into a gel at body temperature and provide a scaffold for cell proliferation. Injectable scaffolds that harden *in situ* can reduce the invasiveness of implantation procedures.[10]

Since direct cell injection is minimally invasive, it is the clinically preferred method of cell transplantation; however, this procedure often results in low cell viability due to membrane disruption during injection.[11] When cells undergo injection, the three major forces they are subjected to are pressure drop across the cell, shearing forces due to linear shear flow, and stretching forces due to extensional flow when flowing from the wide syringe into the skinnier needle; the leading cause of cell death is extensional flow.[11] Aguado et al. found that alginate hydrogels protect cells from the damage of extensional flow and increase cell viability for injected cells.[11]

In this study, we chose pluronic F-127 poloxamer as a hydrogel to determine its effect on cell viability during ejection from a syringe needle. Multiple weights, as well as different concentrations of carbon nanobrushes (CNBs), were used. Marks et al. found that the hydrogel is functional as a tissue scaffold for multiple cell lines[12,13] and that CNBs can be successfully dispersed into pluronic F-127 poloxamer gels and still allow cardiac fibroblasts and myocytes to proliferate, in addition to creating electrically conductive hydrogels for tissue regeneration.[9] It was noticed that there was a lack of a cytotoxic response to both the poloxamer and the CNB content.[9] Thus, using these gels with CNBs allows for electrically conductive scaffolds that harden *in situ* and protect cells from the mechanical forces experienced during syringe needle injection. This work has relevance for tissue engineering and tissue regeneration in clinical medicine.

II. MATERIALS AND METHODS

A. Materials and Equipment

Materials and equipment were as follows: Pluronic F-127 poloxamer (Sigma-Aldrich, St. Louis, MO), CNBs (Chemistry Department, University of Rhode Island, Kingston, RI), D1 multipotent mouse bone marrow stromal precursor cells (ORL UVA), Trypan Blue solution (Sigma-Aldrich), phosphate-buffered saline (Sigma-Aldrich), syringe pump, load cell, ARG2 rheometer (TA Instruments, New Castle, DE), light microscope, and the COMSOL software package (Comsol, Palo Alto, CA).

B. Construction of CNBs

The CNBs were constructed by coating carbon nanotubes with a polyaryl polymer brush. The size of the CNB ranged from 5 to 20 μm in length. The diameter of the CNB was 15–30 nm. The CNBs were negatively stained with phosphotungstate.

C. Preparation of Poloxamer Solution

Two forms of poloxamer solution were prepared: 30 wt% and 35 wt%. First, pluronic F-127 poloxamer was dissolved in 4°C deionized, degassed water to make a 30 wt% solution while continually mixing with a magnetic stir bar and stir plate. The solution was mixed for approximately 10 minutes at a time before it was placed back into a 4°C refrigerator to retain its temperature of 4°C. The solution was stirred multiple times and then placed in a 4°C refrigerator overnight to remove bubbles and fully liquefy the solution. The following day, the solution was mixed using a magnetic stir bar and plate and was again allowed to sit overnight. This process was repeated until all of the pluronic F-127 was dissolved and the solution was completely clear. Then, the same procedure was used to create a 35 wt% poloxamer solution, using the appropriate amount of pluronic F-127 poloxamer.

D. Preparation of Poloxamer Hydrogels with CNBs

The poloxamer hydrogels were prepared by pipetting 5 mL of solution into individual wells of a six-well plate. To create 0 vol% CNB gel samples, the well was left untouched. To create 0.1 vol% CNB, 0.5 vol% CNB, 1 vol% CNB, and 5 vol% CNB gel samples, 5 μL, 25 μL, 50 μL, and 200 μL of CNBs were added, respectively. Once the CNBs were deposited, the plate was gently swirled to

uniformly mix the CNBs into the hydrogels, and the six-well plates were placed in a 4°C refrigerator for at least 20 minutes to fully re-liquefy. This procedure was repeated for many samples of both 30 wt% and 35 wt% poloxamer hydrogels.

E. Rheology of Hydrogels

1. Dynamic Oscillatory Rheology

Experiments were performed on an ARG2 rheometer (TA Instruments) at 25°C with a humidity chamber. All samples were characterized using conical plate geometry (40 mm diameter, 1°0'26" cone angle) with frequency sweeps from 0.1 to 100 s^{-1} and a shear strain of 5% (n=3).

2. Linear Shear Rheology

Experiments were performed on an ARG2 rheometer (TA Instruments) at 25°C with a humidity chamber and conical plate geometry (40 mm diameter, 1°0'26" cone angle). First, a linear shear rate sweep from 10 to 8686 s^{-1} (max of ARG2) was applied. Shear-thinning experiments were performed by applying a constant linear shear rate of 5.3 s^{-1} for 100 s, then applying a constant high shear rate of 8686 s^{-1} (max of ARG2) for 300 s, and finally allowing the hydrogel to recover by applying a shear rate of 5.3 s^{-1} for 100 s (n=3).

F. Pressure Measurements During Syringe Needle Flow

All samples, including deionized water as a control (approximately 3 mL of each), were loaded into 3-mL syringes (diameter = 8.66 mm) with a 27-gauge one-half-inch needle. This was done by taking out the syringe plunger and pipetting the hydrogel into the syringe, since it is too thick to draw up through the needle. The loaded syringe was then mounted on a syringe pump with a compression load cell fitted directly between the syringe pump pusher block and the syringe plunger. The ejection was done at a constant flow rate of 1000 μL/min in a 4°C refrigerator (n=3). The peak ejection pressure is calculated as the peak force during ejection divided by the inner cross-sectional area of the syringe. The pressure drop is determined as the difference between the entrance pressure applied at the plunger and the exit pressure at the needle opening (atmospheric pressure).

G. Cell Culture

Multipotent mouse bone marrow stromal precursor cells (D1) were cultured in cell culture flasks using Dulbecco's modified Eagle's medium (Invitrogen, Carlsbad, CA) containing 10% fetal bovine serum and 1% penicillin-streptomycin solution. Passages 14–18 were used for experiments. All cells were cultured in a humidified 5% CO_2 incubator at 37°C.

H. Cell Encapsulation and Syringe Needle Flow Viability Assay

Cells were resuspended in cell media (same formulation as used for cell culture) at a density of approximately 1.8×10^6 cells/mL. The cell suspension (0.5–1 mL) was then seeded onto 5 mL-deionized water and 5 mL-hydrogel samples of 30 wt% gel/0 vol% CNB and 35 wt% gel/0 vol% CNB (n=3) that had been previously placed in the incubator to solidify. Initial cell viability was determined using a Trypan Blue LIVE/DEAD Assay (Invitrogen). The sample (3 mL) was then loaded into a 3-mL syringe with a 27-gauge one-half-inch needle. This was done by taking out the syringe plunger and pipetting the hydrogel into the syringe, since it is too thick to draw up through the needle. The loaded syringe was then mounted on a syringe pump and ejected at a constant flow rate of 1000 μL/min. After ejection, cell viability was determined using a Trypan Blue LIVE/DEAD Assay. The ejected samples were then placed in the incubator for 24 hours, and cell viability was again assessed using a Trypan Blue LIVE/DEAD Assay. Samples were imaged using a light microscope**.**

I. Linear Shear Flow

Using conservation of mass, during flow through a syringe needle, the volumetric flow rate (1000 μL/min) is constant through the constriction point between the syringe and needle ($d_{syringe}/d_{needle} \sim 41$). The linear fluid velocity within the syringe and needle can be determined with the following equation:

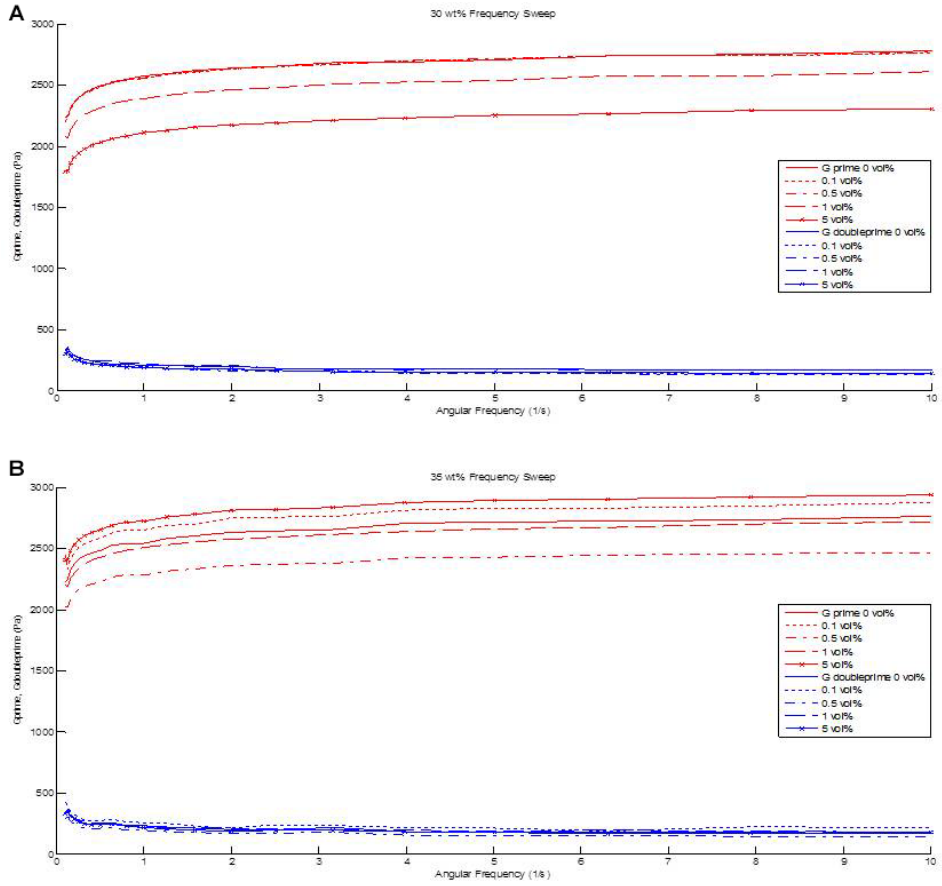

FIG. 1: Rheology of pluronic F-127 poloxamer. Storage (G') and loss (G") moduli of 30 wt% (A) and 35 wt% (B) gel samples.

$$v = \frac{Q}{\pi r^2}$$

where v is the velocity, Q is the volumetric flow rate (1000 µL/min), and r is the inner radius. For the syringe, r = 4.33 mm and $v_{syringe}$ = 0.283 mm/s. For the needle, r = 0.104 mm and v_{needle} = 490.5 mm/s. Thus, the linear fluid velocity in the needle is 1733 times greater than that of the syringe.

The shear rate at the wall of a pipe for a Newtonian fluid (γ') is given by the following equation[11]:

$$\gamma' = \frac{4v}{r}$$

This leads to a linear shear rate of 0.2614 s^{-1} in the syringe and 18,865 s^{-1} in the needle. A rheometer was used to apply a shear rate of 8686 s^{-1} (its maximum value) for 5 seconds, even though the actual flow time for the cells to pass through a 12.7-mm-long needle is as follows:

$$t = \frac{d}{v} = \frac{12.7\ mm}{490.5\ mm/s} = 0.026\ s.$$

J. Finite Element Simulation of Syringe Needle Flow

Fluid flow simulation was conducted in COMSOL 4.0a (Comsol) using a mesh of free tetrahedral elements and boundary conditions of 1000 µL/min at the inlet and atmospheric pressure at the outlet. An incompressible Newtonian fluid model was used to simulate the flow of pluronic F-127 poloxamer at 4°C.

TABLE 1: Properties of various types of pluronic F-127 poloxamer

Weight %	Volume %	Viscosity (Pa*s)[a]	Viscosity (cP)[a]	Plateau G' (Pa)[b]	Plateau G'' (Pa)[b]	Pressure Drop (kPa)	SD for Pressure Drop	Sample Size (n)
30	0	6.41E-04	6.41E-01	2778	352.9333	546.5744	0.486129	3
	0.1	5.26E-04	5.26E-01	2762	335.9333	586.1412	60.25502	3
	0.5	6.69E-04	6.69E-01	2764	321.3333	567.3903	239.1269	3
	1	4.75E-04	4.75E-01	2609.7	348.7333	577.5467	172.5228	3
	5	6.09E-04	6.09E-01	2299.7	308.5667	580.3212	124.5967	3
35	0	4.81E-04	4.81E-01	2757.3	363.4	1237.714	217.5908	3
	0.1	3.00E-03	3.00E+00	2872	452.4333	1407.433	206.0075	3
	0.5	5.85E-04	5.85E-01	2462.7	302.7	1731.833	24.90147	3
	1	5.98E-04	5.98E-01	2717.3	354.8667	1880.033	291.7226	3
	5	6.63E-04	6.63E-01	2934	347.45	1810.533	96.09476	3
Water						2.6236	26.50098	3

[a]Viscosity values were obtained at a shear rate of 8686 s^{-1}.
[b]Plateau values are taken within angular frequencies of 1–10 s^{-1}.

III. RESULTS

A. Rheology of Hydrogels

Using oscillatory shear rheometry, pluronic F-127 poloxamer samples of all weights and CNB concentrations had higher storage moduli (G') than loss moduli (G''), indicating that the materials are hydrogels, as expected (Fig. 1). For the 30 wt% gels, increasing CNB concentration caused a decrease in G'. There was not as clear of a trend for 35 wt% gels, although the highest CNB concentration samples did have the largest G', whereas 0 vol% CNB samples had the lowest G'. The plateau G' and G'' values with an angular frequency range of 1–10 s^{-1} are reported in Table 1 for all samples.

Viscosity measurements at the 8686 s^{-1} shear rate are also reported in Table 1. The peak hold step graphs from which the data were taken are displayed in Fig. 2. At a shear rate of 5.3 s^{-1}, similar to the shear experienced within the syringe during ejection at a flow rate of 1000 μL/min, the linear viscosity was very unstable for all samples. It tended to increase over time during this shear-thinning step. When the shear rate was increased to 8686 s^{-1}, the viscosity dropped for all samples and remained constant throughout. This increased shear rate mimics the shear that the gel experiences when flowing through the needle at a flow rate of 1000 μL/min. Once the shear rate was reduced to 5.3 s^{-1} again, the viscosity again increased with time although there was much instability again.

B. Pressure Drop Measurements

The pressure drop results from injecting the hydrogel samples through a 3-mL syringe with a 27-gauge one-half-inch needle are summarized in Table 1. The pressure used to calculate the pressure drop is taken from the peak value of the force profile. A sample force profile of this injection is included in Fig. 3. When the syringe is first pushed against the gel, the force profile immediately begins to increase rapidly until it reaches a maximum value. This maximum value is the force required to initiate flow. After flow is initiated, the force then decreases slightly and levels off, after which it remains practically constant throughout the rest of the ejection process. The pressure drop required to inject pluronic F-127 poloxamer through a syringe at all CNB concentrations is much higher than that required to push water through a syringe, as expected, but this increased pressure drop comes along with extra protection of the cells. The difference in pressure

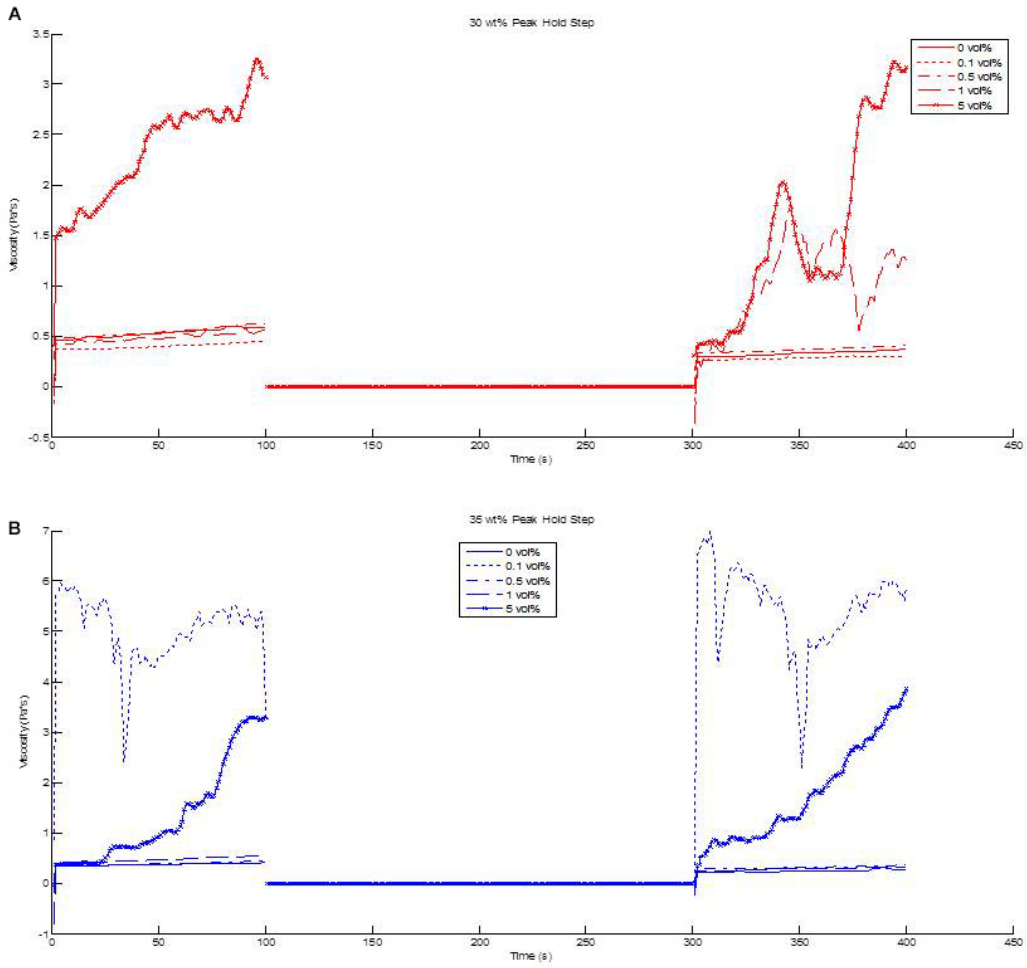

FIG. 2: Shear-thinning and recovery behavior of pluronic F-127 poloxamer. Shear-thinning and recovery behavior of 30 wt% (A) and 35 wt% (B) gel samples.

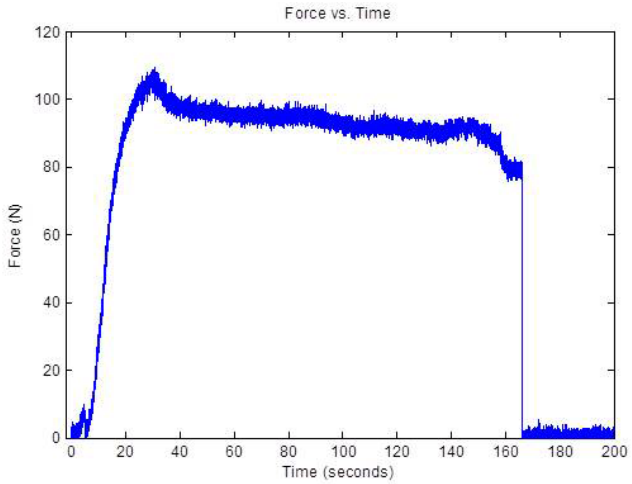

FIG. 3: Sample force profile of pluronic F-127 poloxamer injection through a 3-mL syringe with a 27-gauge one-half-inch needle. 35 wt% gel/0.5 vol% CNB sample data.

TABLE 2: Statistical analysis of differences in pressure drop between water, 30 wt%, and 35 wt% gels

	30 wt% gel/ 0 vol% CNB	35 wt% gel/ 0 vol% CNB
Water	0.000393712	0.004691332
30 wt% gel/ 0 vol% CNB	—	0.015743209

Data are presented as *P* values for one-tailed t-tests.

drop is significant for water and all CNB concentrations of 30 wt% gels ($P<0.0267$). In addition, the pressure drop for water and all CNB concentrations of 35 wt% gels was also significant ($P<0.00469$). The pressure drop for 35 wt% gels is much greater than that required for 30 wt% gels for all CNB concentrations ($P<0.0157$), as would be expected since 35 wt% gels are much thicker.

There are mixed results as to whether CNB concentration significantly affects pressure drop. Within 30 wt% gels, a comparison of the different CNB concentrations suggests that changing the CNB concentration does not significantly affect pressure drop for 30 wt% gels ($P>0.186$). This same result is seen for the 35 wt% gel/0 vol% CNB and 35 wt% gel/0.1 vol% CNB samples ($P=0.191$). However, a comparison of the higher CNB concentrations of 35 wt% (0.5 vol%, 1 vol%, and 5 vol%) to 35 wt% gel/0 vol% CNB suggests that the increase in pressure is significant ($P<0.0287$). Between 0.5 vol%, 1 vol%, and 5 vol%, there is no significant difference between these three ($P>0.235$). The effect of CNB concentration on pressure drop seems to be dependent on the weight of the gel. All *P* values listed above are for one-tailed t-tests, and *P* values showing comparisons between water and the two gel weights are listed in Table 2.

C. Syringe Needle Flow Viability

When D1 cells were subjected to syringe needle flow in deionized water as a control, the viability dropped dramatically during syringe needle flow. From preinjection to postinjection, the change in cell viability was −76.14% ($n=3$). From preinjection to 24 hours postinjection, the change in cell viability was −73.67% ($n=3$). When the cells were encapsulated in pluronic F-127 poloxamer, however, viability was maintained, suggesting that these gels are capable of protecting cell membranes from bursting under the mechanical stresses applied by syringe needle flow. For 30 wt% gel/0 vol% CNB gels, the cell viability change from preinjection to postinjection was only −19.38%, whereas it was −38.21% for 24 hours postinjection. Compared with water for postinjection ($P=0.00186$) and for 24 hours postinjection ($P=0.0285$), our results suggest that the 30 wt% gel improves cell viability significantly. The 35 wt% gel/0 vol% CNB gels protected the cells even more successfully. The cell viability change from preinjection to postinjection was 0.51%, and it was −19.37% for 24 hours postinjection. The *P* value for the comparison of preinjection to postinjection cell viability was 0.00162; the *P* value for the comparison of preinjection to 24 hours postinjection cell viability was 0.0125.

While the 35 wt% gel seemed to increase cell viability compared to the 30 wt% gel, the results were not statistically significant. The *P* value between 30 wt% and 35 wt% gels postinjection was 0.0772, whereas it was 0.150 at 24 hours postinjection. These results suggest that the hydrogels certainly provide protection for the cells during syringe needle flow to prevent the cell membrane from bursting, increasing cell viability. However, as the thickness of the gel increases, its ability to protect cells from bursting in syringe needle flow is not statistically significant. The results of the syringe needle flow viability tests are summarized in Table 3, with *P* values from one-tailed t-tests displayed in Table 4. The results are also shown graphically in Fig. 4. Pictures of the cells are shown in Fig. 5.

D. Finite Element Simulation of Syringe Needle Flow

The COMSOL model results are shown in Figs. 6 and 7. Velocity slices throughout the entire syringe are shown in Fig. 6A, while the velocity of the needle is shown in Fig. 6B. The model shows that the greatest velocity occurs in the center of the needle, as expected for flow through a cylinder, which will have a parabolic velocity profile. The pressure also drops as the gel flows through the needle, which is

TABLE 3: Syringe needle flow cell viability

Weight %	Volume %	Sample	Preinjection			Postinjection				24 Hours Postinjection			
			Total Count[c]	Live Count[c]	Cell Viability (%)	Total Count[c]	Live Count[c]	Cell Viability (%)	Viability Change (%)[a]	Total Count[c]	Live Count[c]	Cell Viability (%)	Viability Change (%)[b]
30	0	1	3.35E+06	1.75E+06	52.24	2.08E+06	5.00E+05	24.10	-28.14	1.23E+06	1.75E+05	14.29	-37.95
		2	1.05E+06	4.25E+05	40.48	9.00E+05	3.00E+05	33.33	-7.14	5.00E+05	1.00E+05	20.00	-20.48
		3	5.00E+05	4.00E+05	80.00	5.25E+05	3.00E+05	57.14	-22.86	5.25E+05	1.25E+05	23.81	-56.19
		Average							-19.38				-38.21
		SD							10.92				17.86
35	0	1	8.75E+05	5.50E+05	62.86	3.25E+05	2.50E+05	76.92	14.07	3.50E+05	1.25E+05	35.71	-27.14
		2	8.50E+05	4.75E+05	55.88	6.25E+05	3.75E+05	60.00	4.12	2.50E+05	1.50E+05	60.00	4.12
		3	6.75E+05	4.50E+05	66.67	7.00E+05	3.50E+05	50.00	-16.67	4.75E+05	1.50E+05	31.58	-35.09
		Average							0.51				-19.37
		SD							15.68				20.73
Water		1	2.00E+05	2.00E+05	100.00	2.00E+05	7.50E+04	37.50	-62.50	3.75E+05	5.00E+04	13.33	-86.67
		2	5.50E+05	5.25E+05	95.45	4.25E+05	5.00E+04	11.76	-83.69	6.25E+05	1.25E+05	20.00	-75.45
		3	4.50E+05	4.00E+05	88.89	3.75E+05	2.50E+04	6.67	-82.22	2.50E+05	7.50E+04	30.00	-58.89
		Average							-76.14				-73.67
		SD							11.83				13.97

[a]Viability change = cell viability (postinjection) – cell viability (preinjection).
[b]Viability change = cell viability (24 hours postinjection) – cell viability (preinjection).
[c]All counts are in cells/mL and were measured using a hemacytometer.

TABLE 4: Statistical analysis of differences in cell viability between water, 30 wt%, and 35 wt% gels

	Postinjection		24 Hours Postinjection	
	30 wt% gel/ 0 vol% CNB	35 wt% gel/ 0 vol% CNB	330 wt% gel/ 0 vol% CNB	35 wt% gel/ 0 vol% CNB
Water	0.001860154	0.0016178	0.028474976	0.012490192
30 wt% gel/0 vol% CNB	—	0.077195828	—	0.150156485

Data are presented as *P* values for one-tailed t-tests.

also expected. Fig. 7A shows the pressure slices of the entire syringe, while Fig. 7B shows the pressure variation throughout the needle. A schematic of the flow through the syringe and needle, modeled after Fig. 2A of Aguado et al.,[11] is displayed in Fig. 8.

IV. DISCUSSION

Our results demonstrate that pluronic F-127 poloxamer hydrogels are protective of cells during syringe needle flow. Based on the results of the syringe needle flow viability portion of this study, 30 wt% and 35 wt% hydrogels cause less of a decrease in cell viability from preinjection to postinjection than simply suspending the cells in water. As previously discussed, cells experience three major types of forces during injection: pressure drop across the cell, shearing forces due to linear shear flow, and stretching forces due to extensional flow; extensional flow has been reported to be the leading cause of cell death during injection.[11]

Shear forces on the hydrogels were considered in the rheological portion of this study. The maximum shear rate during syringe needle flow occurs at the wall of the needle and was calculated to be 18,865 s^{-1}. A shear rate of 8686 s^{-1} (the maximum shear rate of the rheometer) was applied to the hydrogels to determine its effect on the gels (see Table 1 and Figs. 1 and 2). Aguado et al. previously determined that these forces are not responsible for the majority of cell deaths in syringe needle flow,[11] so we next assessed the pressure drop associated with the gels.

The peak pressure drop for the 30 wt% gels was 586.1412 kPa, while the peak pressure drop for the 35 wt% gels was 1880.033 kPa (see Table 1). These values are larger than those found by Aguado et al. for alginate gels[11]; however, the large

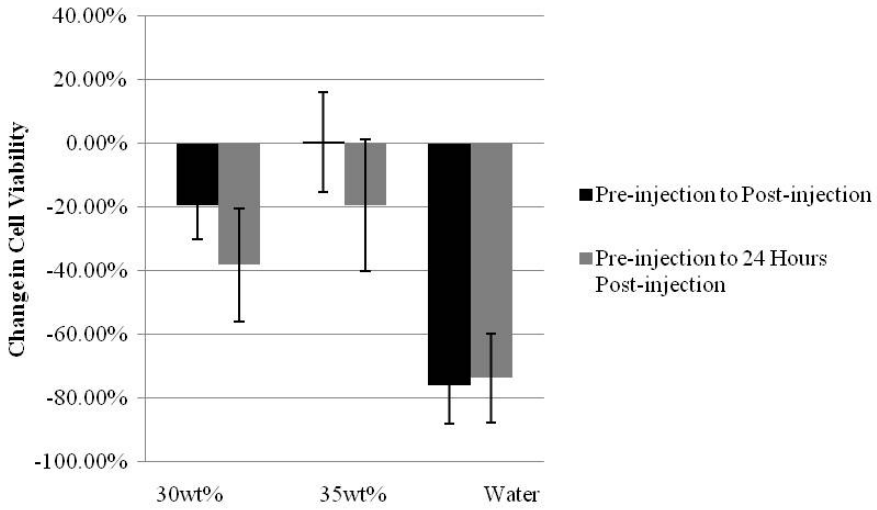

FIG. 4: Syringe needle flow cell viability results. All results shown are the average of three trials (*n*=3), and all hydrogels are 0 vol% CNB. Error bars show 1 standard deviation.

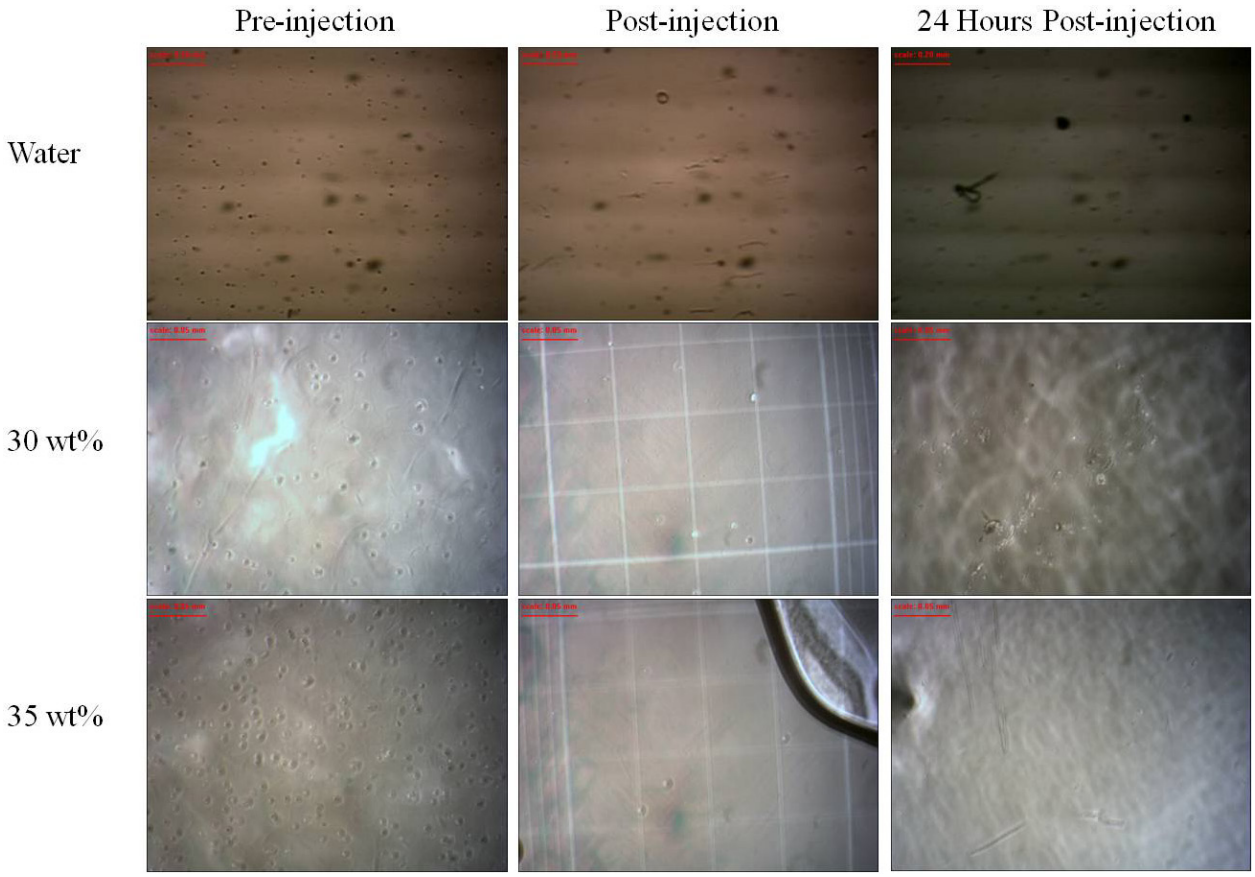

FIG. 5: Sample pictures of cells suspended in water and the hydrogels prior to injection, immediately after injection, and 24 hours after injection. All of the hydrogels for these pictures are 0 vol% CNB. The postinjection pictures for the hydrogels were taken with the cells on a hemacytometer.

pressure drop did not seem to negatively impact the ability of the gels to protect the cells, most likely because the gels act as a protective scaffold for the cells. Despite having a higher pressure drop, the 35 wt% gel had the highest cell viability. This suggests that pressure drop is not the major factor attributing to cell death, as expected.

Overall, the purpose of this study was to determine whether pluronic F-127 poloxamer hydrogels affect cell viability during syringe needle flow. Having shown that they do indeed increase cell viability, it is necessary to discuss the mechanical properties of the gels that allow for this protection. One possible reason for the protective quality of the hydrogels is discussed in detail by Aguado et al., and it is known as "plug flow".[11] In plug flow, a layer of the hydrogel along the walls of the needle may undergo shear-thinning and becomes more fluid as a result.[11] This causes it to act as a lubricant for the rest of the gel, allowing the gel in the middle to flow freely through the needle.[11] In turn, the hydrogels are able to protect cells from bursting during syringe needle flow.

V. CONCLUSION

Cell transplantation could be very easy and minimally invasive for patients by using syringe needle injection; however, low cell viability has been a major problem with this method in the past. By encapsulating cells in hydrogels, cell viability can be increased, making this method more suitable for clinical use. This work demonstrates that poloxamer F-127 of both 30 wt% and 35 wt% improves cell viability during syringe needle flow. In addition, the presence of CNBs does not affect cell viability. These results are significant for tissue regeneration.

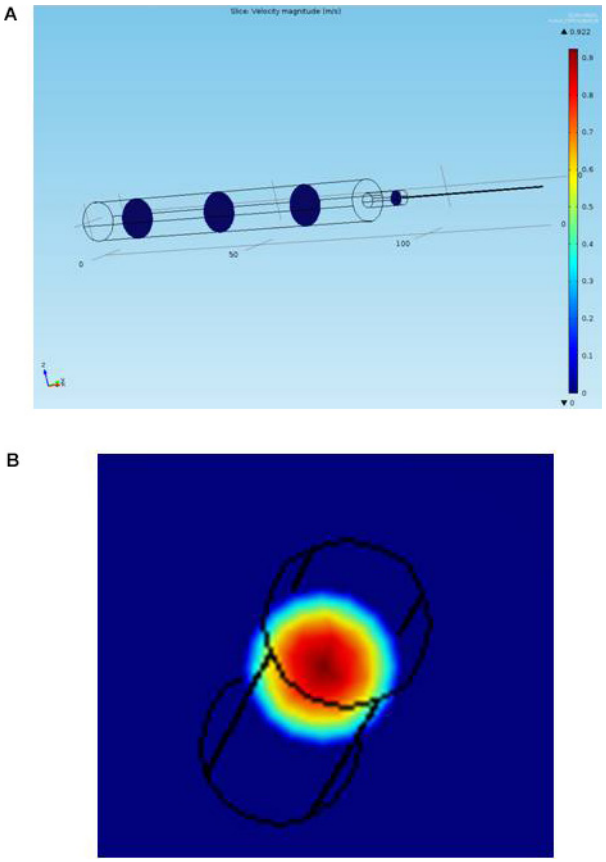

FIG. 6: COMSOL velocity model of syringe needle flow. Velocity slices of the entire syringe and needle (A) and just the needle (B). The velocity color profile for B is the same as that shown for A.

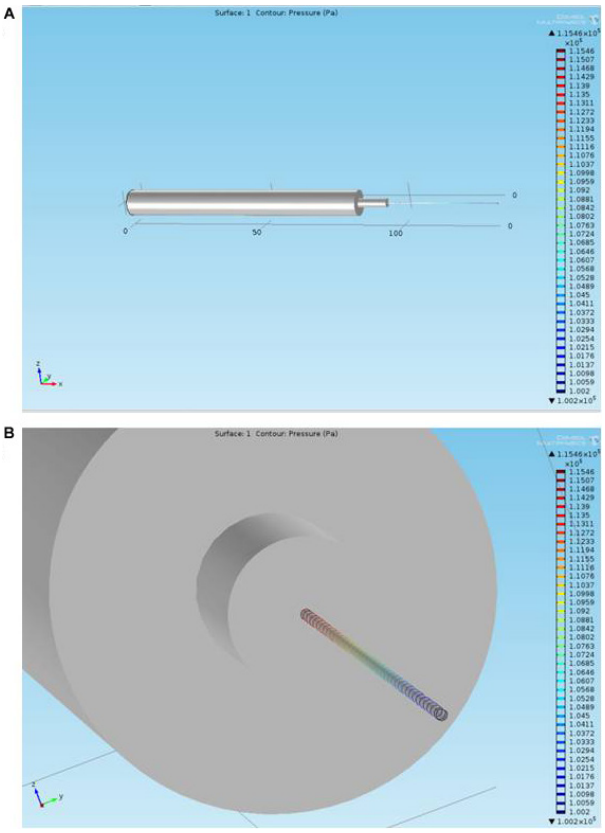

FIG. 7: COMSOL pressure model of syringe needle flow. Pressure slices of the entire syringe and needle (A) and just the needle (B).

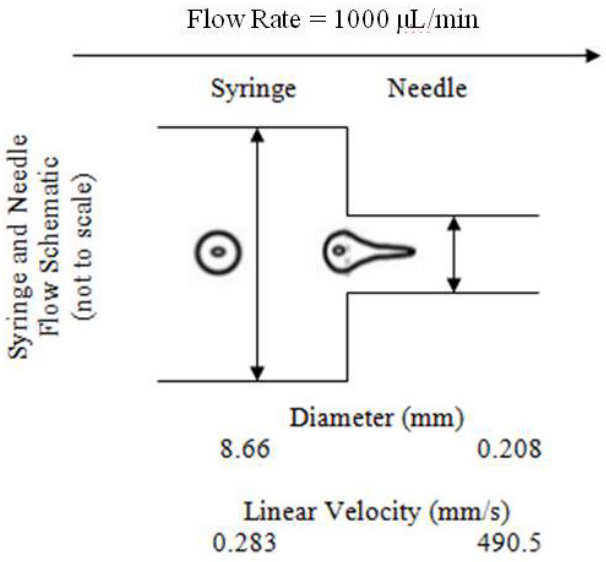

FIG. 8: Flow characteristics during syringe needle flow. Schematic of syringe and needle (not drawn to scale). Data are based on Fig. 2A from Aguado et al.[11]

With hydrogels that have reverse thermodynamic properties, the cells can be suspended in the gel in solution form and injected through a syringe needle. The gel will then solidify at body temperature, forming a scaffold for cell proliferation. The addition of CNBs to the gels can also allow for these scaffolds to be electrically conductive. Future studies will determine the ability of this hydrogel to generate tissue growth *in vivo* through syringe needle injection of cells encapsulated in poloxamer F-127 into an animal hide and eventually live animals. This process for cell transplantation is very promising and could be useful in tissue engineering and tissue regeneration in clinical medicine.

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