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Contents

Water Soluble Polymers for Immunoisolation I: Complex Coacervation and Cytotoxicity

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Seventy five synthetic, semi-synthetic, natural and biological water soluble polymers have been evaluated as potential biomaterials for cell and islet immunoisolation. Measurements have included the cytotoxicity of polyanion and polycation solutions towards insulinoma cells as well as the type of complex coacervate interaction produced. These results have been coupled with metrics delineating the quality of the capsular membrane produced and correlated with molecular properties of the individual polymers tested. Microcapsules prepared from over one thousand binary polyelectrolyte combinations have been characterized according to their mechanical strength, capsule shape, surface smoothness, stability, and swelling or shrinking. Based on this screening 47 pairs have been identified as alternatives to the standard poly-L-lysine-alginate chemistry. The quality of the membrane produced was observed to be a strong function of the polymer molecular weight, as well as the solution concentration. Additionally, the ionic content of the backbone, the chemistry and location of functional group attachment, the chain rigidity, aromaticity, conformation and extent of branching were identified as important variables in the type of complex produced. The presence of secondary hydrogen bonding interactions was also found to be significant. Processing conditions such as the type and concentration of the simple electrolyte, the pH, the reaction time and surface coating have also been investigated.

Keywords: Bioartificial pancreas, biomaterials, complex coacervation, immunoisolation, microencapsulation, polyelectrolytes, water soluble polymers.

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1 Introduction

Water soluble polymers include naturally occurring polysaccharides [1], biomolecules such as DNA, semi-synthetic species such as modified cellulose, as well as synthetic molecules, predominantly based on radical polymerization of acrylic monomers [2]. At present their principal applications are as hydrocolloids in food additives [3], in environmental applications such as municipal water treatment [4] and for resource recovery and processing [5]. The market for water soluble polymers is now several billion dollars per annum, with growth rates in consumption of 5–8% exceeding that of most sectors in the chemical industry. Over the past thirty years, considerable research interest has been dedicated to the utilization of water soluble and swellable polymers in biological applications. These include opthalmological devices [6], matrices for controlled drug delivery [7, 8], dental materials and scaffolds for tissue regeneration [9, 10]. They can also be utilized for the formation of immunoisolation barriers [11]. The latter involves the production of semi-permeable membranes by either a phase inversion process [12] or a complex coacervation reaction [13].

The principal issues involved in developing polymeric biomaterials are biodegradability and biocompatibility. While degradation can be quantified relatively precisely [14], a definition of biocompatibility has been elusive. At present, one can only refer to the suitability of a material for a specific application in a given site within the body. Furthermore, polymers which will contact blood have much more stringent requirements since they can often provoke a stronger immune system response. Unfortunately some polymers which have shown good compatibility, such as polyethylene oxide, have very poor mechanical properties. To compensate for this, two general approaches are employed. In some instances, mechanically suitable copolymers have been used to produce devices such as an artificial heart [15, 16] and are then surface coated to attempt to prevent a host system response [17]. The major limitation in this regard is the difficulty in obtaining complete surface coverage and the reversibility of adsorption. An alternative approach is to synthesize biomaterials from polymers which have intrinsically good biocompatibility, for the purpose at hand, and to avoid the necessity of coating. It is this latter philosophy to which the authors of this paper subscribe. Therefore we have been motivated to evaluate both the material properties and compatibility of polyelectrolytes as perspective immunoisolation barriers.

Several competing strategies for immunoisolation such as vascular grafts [18], hollow fibres [19] and both macro- [20, 21] and microencapsulation [22–24] have been evaluated over the past two decades. These have been discussed in several recent reviews [25, 26]. The primary advantages of microencapsulation are that it avoids the necessity of major surgery, and the use of a complex coacervation reaction facilitates the investigation of alternative polymer chemistries. The separation of cells into several thousand particles also provides additional security in that some microcapsules can fail, or be rejected, without subjecting the entire population to risk. The application of polymers as immunoisolation barriers includes the development of a bioartificial liver [27, 28] and bioartificial parathyroid [29]. Water soluble or swellable macromolecules are also used for pain control for terminal cancer patients [30], in the treatment of Alzheimer's [31] and neurological disorders [32], and in the encapsulation of pancreatic islets.

The development of biological microencapsulation systems has included pioneering efforts by Chang [33], Lim and Sun [34] and Sefton and Broughton [35]. The latter two have focused on the immunoisolation of pancreatic islets for the formation of a bioartificial pancreas. Thin film polymer membranes comprised of water-insoluble thermoplastics, symplexes and hydrogel copolymers have been prepared, and several recent reviews detail the technological aspects involved in cell or islet encapsulation [36–38]. Unfortunately the fragile nature of islets, and the specificity of the capsule processing conditions to the properties of the often viscoelastic polymer fluid, have limited the number of polymers which have been rigorously evaluated (Table 1). Indeed, most researchers have been limited to the poly-L-lysine-alginate [35] and alginate-chitosan [55] systems which are based on the ionotropic gelation of alginate with polyvalent cations, typically calcium. However, although lysine-alginate produces quite stable membranes, it has relatively poor mechanical properties. Ionotropic gelling alterna-

Table 1. Summary of nonionic and ionogenic water soluble polymers utilized for encapsulation

Membranes Prepared Via Coacervation		Gelling Agent/	Ref.
Inner Polymer (Core)	External Polymer (Receiving Bath)	Template	
Alginate	Polyvinylamine	Calcium	39
Alginate	Polyvinylamine	Calcium	40
Alginate	Protamine	–	41
Alginate	Spermine	–	42
Alginate	Polybrene	Barium	43
Cellulose Sulfate	Polydiallyldimethyl ammonium chloride	–	44
Carboxymethylcellulose	Chitosan	–	45
Carboxymethylcellulose	Diethylaminoethyldextran	–	45
Carrageenan-κ	Chitosan	Potassium	46
Chitosan	Alginate	Calcium	47
Chitosan	Pentasodiumtripoly-phosphate hexahydrate	–	48
Chitosan	Xanthan	–	49
Chondroitin Sulfate A	Chitosan	–	45
Chondroitin Sulfate C	Spermine	–	43
Heparin	Protamine	–	50
Hyaluronic Acid	Chitosan	–	45
Pentasodiumtripoly-phosphate hexahydrate	Chitosan	–	51
Polyacrylates/Methacrylates (anionic)	Polyacrylates (cationic)	–	52
Polyphosphazene (anionic)	Polylysine	Calcium	53
Polystyrene Sulfonate	Polybrene	Agarose	54

tives for alginate, as an inner polymer, have thus far been limited to the cationic chitosan and blends of alginate with other polysaccharides such as carrageenan, carboxymethylcellulose or dextran sulfate [56]. Furthermore, it has been speculated that a family of capsule chemistries will need to be available in order to provide alternatives in the event that the primary immunoisolation material is rejected by a given patient. This problem is likely to be particularly acute for Type-I diabetics, since they typically contract the disease for over 40 years. Therefore, in an attempt to identify alternatives to the classical systems listed in Table 1, we have undertaken a massive screening of polyelectrolytes in an attempt to make molecular inferences as to the complexation mechanism. The evaluation has included 35 polyanions and 40 polycations in 1235 binary combinations (Table 2).

Table 2. Polyelectrolytes utilized in this screening

#	Polymer type and molecular weight grade (if applicable)	Brand name	Concentration tested (wt %)	Supplier
	Naturally occurring polyanions			
1a	Alginate (Sodium), High	Keltone HVCR	0.2–2.0	Kelco/Merck, San Diego, CA
1b	Alginate (Sodium), Low	Keltone LV	0.2–2.0	Kelco/Merck, San Diego, CA
1c	Alginate (Sodium), Low	Manugel DMB	0.2–2.0	Kelco/Merck, San Diego, CA
1d	Alginate (Sodium), Low–Medium–High	–	0.2–2.0	Sigma, St. Louis, MO
1e	Alginate (Sodium), Low	UP LVG	0.2–2.0	Pronova Biopolymer, Drammen, Norway
1f	Alginate (Sodium) Medium	UP MVG	0.2–2.0	Pronova Biopolymer, Drammen, Norway
1g	Alginate (Sodium), High–Low	Kelcoloid HVF-LVF	0.2–2.0	Kelco/Merck, San Diego, CA
2	Alginate (Proplylene Glycol Modified), Medium–High	Protanal SD-H, PVH-A	1.0–2.0	Pronova Biopolymer, Drammen, Norway
3	Carboxymethyl Amylose	–	0.5–2.0	Sigma, St. Louis, MO
4a	Carboxymethyl Cellulose (Sodium), Low–Medium–High	–	0.5–2.0	Sigma, St. Louis, MO
4b	Carboxymethyl Cellulose (Sodium), Medium	7MF	0.5–1.5	Aqualon/Hercules, Wilmington, DE
5	Carboxymethyl Dextran	–	1.0–15.0	Fluka, Ronkonkoma, NY
6a	Carrageenan-ι	Gelcarin GP-379 NF	0.2–1.0	FMC Corp., Newark, CT
6b	Carrageenan-κ	Gelcarin GP-911 NF	0.2–1.0	FMC Corp., Newark, CT
6c	Carrageenan-λ	–	0.5–1.5	Fluka, Ronkonkoma, NY
6d	Carrageenan-κ, Low	Aubygel X52	0.5–1.5	Sanofi Bio-Industries, Paris, France
7	Cellulose Sulfate (Sodium)	–	0.2–2.0	Janssen Chimica, Geel, Belgium
8	Chondroitin 4-Sulfate (Sodium)	A	0.2–1.0	Sigma, St. Louis, MO
9	Chondroitin 6-Sulfate (Sodium)	C	0.2–1.0	Sigma, St. Louis, MO
10	Dextran Sulfate, 500 kDa	–	1.0–10.0	Pharmacia, Uppsala, Sweden
11	Gellan Gum (Deacetylated)	Kelcogel	0.6 in 0.3% Hexa-monophosphate	Kelco/Merck, San Diego, CA
12	Gum Arabic	–	1.0	Sigma, St. Louis, MO
13	Heparin, 3 KDa	–	1.0–5.0	Sigma, St. Louis, MO
14a	Hyaluronic Acid, 1–2000 kDa	–	0.1–5.0	Genzyme, Cambridge, MA

Table 2. (continued)

#	Polymer type and molecular weight grade (if applicable)	Brand name	Concentration tested (wt %)	Supplier
14b	Hyaluronic Acid	FCH	0.1–1.0	Pronova Biopolymer, Drammen, Norway
15a	Pectin (Low Esterified)	–	1.0–5.0	Sigma, St. Louis, MO
15b	Pectin (Low Esterified)	315 NHND	1.0–5.0	Sanofi Bio-Industries, Paris, France
16	Polygalacturonic Acid	–	1.0–5.0	Sigma, St. Louis, MO
17a	Xanthan, High	Rhodigel	0.2–2.0	R.T. Vanderbilt, Norwalk, CT
17b	Xanthan, High	Ticaxan	0.2–2.0	TIC Gums, Belcamp, MD
17c	Xanthan, High	Ketrol T/TF	0.5–1.5	Kelco/Merck, San Diego, CA
Synthetic Polyanions				
18	Pentasodiumtripolyphosphate hexahydrate	–	1.0–10.0	Sigma, St. Louis, MO
19	Polyacrylamide (70% Carboxy Modified), 200 kDa	–	1.0–5.0	Polysciences, Warrington, PA
20	Polyacrylamide (90% Carboxy Modified), 200 kDa	–	1.0–5.0	Aldrich, Milwaukee, WI
21	Polyacrylamide-*co*-Acrylic Acid, 10 and 40% Carboxylated	–	1.0–5.0	Polysciences, Warrington, PA
22	Polyacrylamido-2-methyl-1-propanesulfonic Acid	–	1.0–5.0	Aldrich, Milwaukee, WI
23a	Polyacrylic Acid, 2.1,6.10,20,60,140,250, 450 kDa	–	1.0–5.0	Polysciences, Warrington, PA
23b	Polyacrylic Acid, 450,750,1000,4000 kDa	–	0.1–1.0	Aldrich, Milwaukee, WI
23c	Polyacrylic Acid (Modified)	–	0.1–1.0	Gelest, Tullytown, PA
24	Polyglutamic Acid, 5–30 kDa	–	1.0–5.0	Gelest, Tullytown, PA
25	Polymaleic Acid	–	1.0–5.0	Polysciences, Warrington, PA
26	Polymaleic Anhydride	–	1.0–5.0	Polysciences, Warrington, PA
27	Polymethacrylic Acid (Sodium) 15 kDa	–	1.0–2.0	Polysciences, Warrington, PA
28	Polymethylvinylethermaleicacid 20–70 kDa	–	1.0–5.0	Polysciences, Warrington, PA
29	Polymethylvinylethermaleicacid Anhydride, 50, 70 kDa	–	1.0–5.0	Scientific Polymer Products, Ontario, NY

Table 2. (continued)

#	Polymer type and molecular weight grade (if applicable)	Brand name	Concentration tested (wt %)	Supplier
30	Polystyrene Sulfonic Acid (Sodium), 70 kDa	–	1.0–5.0	Polysciences, Warrington, PA
31	Polyvinylphosphate	–	1.0–10.0	Polysciences, Warrington, PA
32	Polyvinylphosphonic Acid	–	1.0–2.0	Polysciences, Warrington, PA
33	Polyvinylsulfone (Anionic)	–	1.0–10.0	Polysciences, Warrington, PA
34	Polyvinylsulfonic Acid (Sodium) 2 kDa	–	1.0–10.0	Polysciences, Warrington, PA
Naturally Occurring or Biological Polycations				
35a	Chitosan Glutamate, Medium	Protasan HV	0.5–2.5	Pronova Biopolymer, Drammen, Norway
35b	Chitosan Glutamate, Low	Protasan LV	0.5–2.0	Pronova Biopolymer, Drammen, Norway
36	Chitosan (Glycol Modified), 80 kDa	–	0.5–2.0	Wako Chemicals, Richmond, VA
37	Dextran (Diethylaminoethyl Modified), 500 kDa	–	1.0–10.0	Pharmacia, Uppsula, Sweden
38	Hydroxyethyl Cellulose Trimethylamine (Quaternary)	JR-125	0.05–0.5	Amerchol, Edison, NY
39	Lysozyme	–	1.0–5.0	Sigma, St. Louis, MO
40	Poly-L-Lysine (Hydrobromide) 30–70 kDa	–	0.1–1.0	Sigma, St. Louis, MO
41	Salmine Sulfate, 5–10 kDa	–	1.0–5.0	Fluka, Ronkonkoma, NY
42a	Protamine Sulfate, 5–20 kDa	Grade III	1.0–5.0	Sigma, St. Louis, MO
42b	Protamine Sulfate	–	1.0–5.0	Fluka, Ronkonkoma, NY
Synthetic Polycations				
43a	Polyacrylamide (Cationic)	492C, 496C	0.05–0.3	Cytec, Wayne, NJ
43b	Polyacrylamide (Cationic)	Jayfloc 3468	0.1–0.5	Callaway, Columbus, GA
44	Polyacrylamide-*co*-Methacryloxyethyltrimethyl-ammonium Bromide, 80/20	–	1.0–5.0	Polysciences, Warrington, PA
45a	Polyallylamine Hydrochloride, 60 kDa	–	1.0–5.0	Polysciences, Warrington, PA
45b	Polyallylamine Hydrochloride, 10, 57 kDa	–	1.0–5.0	Aldrich, Milwaukee, WI
46	Polyamide (Cationic), 100 kDa	Discostrength 5807, Discol 792-A	0.1–0.5	Callaway, Columbus, GA

Table 2. (continued)

#	Polymer type and molecular weight grade (if applicable)	Brand name	Concentration tested (wt %)	Supplier
47	Polyamine	4030	1.0–5.0	Callaway, Columbus, GA
48	Polyamine (Quarternary), dimethylamine/ epichlorohydrin	Agefloc B50	1.0–5.0	CPS Chemicals, West Memphis, AK
49	Polybrene (hexamethrine bromide)	–	1.0–5.0	Sigma, St. Louis, MO
50	Polybutylacrylate-*co*-Methacryloxyethyl Trimethylammonium Bromide (80/20)	–	1.0–5.0	Polysciences, Warrington, PA
51	Poly-3-chloro-2-hydroxypropylmethacryl-oxyethyl dimethylammonium Chloride	–	1.0–5.0	Polysciences, Warrington, PA
52a	Polydiallyldimethylammonium Chloride, Low & High	Agefloc WT and PC Series, Agequat 400	0.5–5.0	CPS Chemical Co., West Memphis, AK
52b	Polydiallyldimethylammonium Chloride, 240 kDa	17338	0.5–5.0	Polysciences, Warrington, PA
53	Polydiallyldimethylammonium Chloride-*co*-Acrylamide, 75/25, 50/50	Agequat C3204, C505, 5008	1.0–5.0	CPS Chemical Co., West Memphis, AK
54	Polydiallyldimethylammonium Chloride-*co*-N-Isopropyl Acrylamide	–	1.0–5.0	Synthesized by R. Pelton, McMaster Univ.
55	Polydimethylamine-*co*-epichlorohydrin (Quaternary), 25,75 kDa	652	1.0–5.0	Aldrich, Milwaukee, WI
56	Polydimethylamine-*co*-epichlorohydrin (Quaternary)	–	1.0–5.0	Scientific Polymer Products, Ontario, NY
57a	Polydimethylaminoethylacrylate-*co*-Acrylamide (Quaternary)	–	0.1–0.5	Synthesized in our laboratory
57b	Polydimethylaminoethylacrylate-*co*-Acrylamide (Quat.), 88/12	–	0.05–0.5	Betz Laboratories, Trevose, PA
58	Polydimethylaminoethylmethcrylate-*co*-Acrylamide (Quat.), 81/19, 9,100 kDa	–	0.05–0.5	Betz Laboratories, Trevose, PA
59	Polydimethylaminoethylmethacrylate (Quaternized)	–	1.0–5.0	Polysciences, Warrington, PA
60	Polydimethylaminoethyl Methacrylate (Acryloxy, Quaternized)	–	1.0–5.0	Polysciences, Warrington, PA

Table 2. (continued)

#	Polymer type and molecular weight grade (if applicable)	Brand name	Concentration tested (wt %)	Supplier
61	Polyethyleneimine, 2,25,40,70,80 kDa	G35 SG, Waterfree SG, Luviquat FC 905/550	0.1–10.0	BASF, Parsippany, NY
62	Polyethyleneimine-Epichlorohydrin Modified, 20 kDa	634	1.0–5.0	Scientific Polymer Products, Ontario, NY
63	Polyethyleneimine (hydroxyethylated), 50,70 kDa	–	1.0–5.0	Polysciences, Warrington, PA
64	Polyethyleneimine (80% ethoxylated), 50,70 kDa	–	1.0–5.0	Scientific Polymer Products, Ontario, NY
65	Poly-2-hydroxy-3-methacryloxypropyl Trimethylammonium Chloride	–	1.0–5.0	Polysciences, Warrington, PA
66	Poly-2-hydroxy-3-methacryloxyethyl Trimethylammonium Chloride	–	1.0–5.0	Polysciences, Warrington, PA
67	Polyhdroxyproplymethacryloxy Ethyldimethyl Ammonium Chloride	–	1.0–5.0	Polysciences, Warrington, PA
68	Polyimadazoline (Quaternary), Oligomer	653	1.0–5.0	Scientific Polymer Products, Ontario, NY
69	Poly-2-methacryloxyethyltrimethylammonium Bromide, 50,200 kDa	–	1.0–5.0	Polysciences, Warrington, PA
70	Polymethacryloxyethyltrimethylammonium Bromide/Chloride	–	1.0–5.0	Polysciences, Warrington, PA
71	Polymethyldiethylaminoethylmethacrylate-*co*-acrylamide 81/19	3200 kDa	0.05–0.5	Betz Laboratories, Trevose, PA
72	Poly-1-methyl-2-vinylpyridinium Bromide, 50 kDa	–	1.0–5.0	Polysciences, Warrington, PA
73	Poly-1-methyl-4-vinylpyridinium Bromide, 50 KDa	–	1.0–5.0	Polysciences, Warrington, PA
74	Polymethylene-*co*-Guanidine Hydrochloride, Oligomer	654	0.2–2.0	Scientific Polymer Products, Ontario, NY
75	Polyvinylamine, 20,70,220 kDa	–	0.1–2.0	Air Products, Allentown, PA
76	Poly-N-vinylpyrrolidone-*co*-Dimethylaminoethyl-methacrylate (Quaternary), High	–	1.0–5.0	Polysciences, Warrington, PA
77	Poly-4-vinylbenzyltrimethylammonium Chloride, 100,400 kDa	707	1.0–5.0	Scientific Polymer Products, Ontario, NY
78	Poly-4-vinylbenzyltrimethylammonium Chloride	–	1.0–5.0	Polysciences, Warrington, PA

1.1
Polymer-Polymer Interactions

Solutions containing two polymers undergo several types of interactions which can ultimately lead to phase separation. These include (a) simple coacervation (incompatibility) which produces two phases of approximately equal volume, and (b) complex coacervation where the polymers are concentrated in a gel or precipitate phase with the supernatant essentially polymer free. The complex coacervation of two charged or nonionic polymers has been shown to be important in membrane formation [57]. In addition to electrostatic effects, secondary interactions such as hydrogen bonding (with a force of 4–6 kcal/mol), van der Waals forces (approximately 1 kcal/mol), as well as charge transfer and hydrophobic interactions can contribute to the stability of the membrane. When one of the polymers is in excess a (c) soluble complex or "sol" is typically formed. The particular nature of the polymer-polymer interaction is dependent on the concentration and density of interacting groups. Complexation is also known to be a function of the molecular weight and solution pH and ionic strength. Generally, polyelectrolytes with high charge densities interact to form precipitates. In most cases, the complex coacervation reaction is stoichiometric beyond a certain chain length (usually a few hundred) [58]. Therefore, the ratio of the interacting species is important. The rate of complexation can be of the order of fractions of a second [59], although the kinetics are reduced with increasing molecular weight. The morphology of the reaction product (precipitate, gel) is also sensitive to the kinetics and time of formation.

2
Experimental

2.1
Identification of Polymers for the Screening

In selecting potential polymers for screening four requirements were established: (1) the polymer must be soluble in water and physiological solutions since organic solvents are, in many cases, cytotoxic; (2) the polymers should have either permanent or pH inducible charges; (3) the primary side chain functional groups should not be known to induce immune system responses; (4) the polymers must either gel in the presence of ions of the opposite charge (chelation) or participate in coacervation reactions. In general, polymers which required additives, such as crosslinking agents, to enhance the membrane formation were not considered. Polymers were selected which contained anionic and cationic charges derived from various functionalities. Additionally, the molecular weight range was varied from oligomeric to several million daltons. Where possible, and in particular for synthetic polymers, the charge spacing within a given polymer was varied to test the effect of charge spacing on the membrane formation. The screening was designed to test an equal number of synthetic and naturally occurring polyanions and polycations. Therefore, approximately twenty candidate

polymers were selected from each of these four categories with the exception of naturally occurring polycations for which relatively few species are readily available.

2.2 Polymer Solution Preparation and Purification

All polymers utilized in this investigation have been listed in Table 2, along with their supplier and the concentration range over which they were tested. Polymers were either used as received or purified by filtration through a 0.22 or 0.45-μm Millipore cellulose acetate membrane. For aseptic applications autoclaving was carried out for 20 min at a temperature of 121 °C. Qualitative properties of each polymer are listed in Table 3. For polymers supplied as solutions, dialysis was carried out in membranes (Spectrum Medical Industries, Houston, TX) with a MWCO of 10,000 daltons.

Polymer solutions were prepared by dispersing the polymer powder in a saline solution prepared with distilled deionized water. Following complete dispersion in the vortex of the fluid the samples were agitated under mild conditions (< 100 RPM) until the solution was homogeneous. For some solutions the dissolution was so rapid that the agitation step could be eliminated. The polymer viscosities were then measured using a Ubbelohde viscometer. The pH of the polymer solutions was adjusted using dilute acetic acid and sodium hydroxide. Some polymers were supplied as liquids and were subsequently diluted with distilled deionized water to the appropriate concentration.

2.3 Polymer Solution Specifications

In order to generate data which could subsequently be utilized for islet encapsulation, specific screening conditions were required. Therefore, all polymer solutions were prepared in a pH range between 5 and 8, a temperature between 20 and 25 °C and an ionic strength which mimicked the physiological solutions required for cell survival. Specifically, the pH was generally kept between 5 and 6 for polycations to permit the dissociation of, for example, tertiary amines. The polyanions, which are generally the preferred candidates for cell suspension fluids, were tested at pHs between 6 and 7 for cell viability reasons. In most cases polymer solutions were prepared by dissolving a powder in phosphate buffer solution (PBS) so as to allow for a convenient osmotic pressure for the cells. Additionally, the viscosities of the two polymeric solutions (nominally one polyanion and one polycation) were kept within a range (<150 cPs) which would be required for the processing of droplets. This generally limited the maximum polymer concentration which could be tested to 1–2 wt % for the polyanions and 1–5% for the polycations, with specific concentrations for all polymers listed in Table 2.