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Fibrillized peptide microgels for cell encapsulation and 3D cell culture

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Abstract

One of the advantages of materials produced by self-assembly is that in principle they can be formed in any given container to produce materials of predetermined shapes and sizes. Here, we developed a method for triggering peptide self-assembly within the aqueous phase of water-in-oil emulsions to produce spherical microgels composed of fibrillized peptides. Size control over the microgels was achieved by specification of blade type, speed, and additional shear steps in the emulsion process. Microgels constructed in this way could then be embedded within other self-assembled peptide matrices by mixing pre-formed microgels with un-assembled peptides and inducing gelation of the entire composite, offering a route towards multi-peptide materials with micron-scale domains of different peptide formulations. The gels themselves were cytocompatible, as was the microgel fabrication procedure, enabling the encapsulation of NIH 3T3 fibroblasts and C3H10T-1/2 mouse pluripotent stem cells with good viability.

Keywords

stem cell; microenvironment; tissue engineering; self-assembly; regenerative medicine

INTRODUCTION

Self-assembled peptide gels have received significant attention as matrices for 3D cell culture and as scaffolds for regenerative medicine.^{1–11} However, one of their significant shortcomings is that they are difficult to manipulate once they have assembled owing to their tendency to fracture, which complicates their use in culture or in vivo. Although some self-assembling peptide gels exhibit the ability to self-heal after they have been subjected to shear forces,^{1, 3, 12, 13} such healing usually takes place only in the absence of excess solvent, which is not the case for many applications in cell culture, in which gels are typically overlaid with culture medium.^{8, 14, 15} Likewise, many tissue sites contain unconfined fluid, including the vasculature, subcutaneous spaces, joint spaces, and spaces between fascial planes. Although it has been shown that self-assembled peptide gels can effectively be used for entrapping or embedding cells,^{3, 4} their susceptibility to fragmentation makes it difficult to transfer cell-laden peptide gels from one container to another, or from culture to an in vivo setting, without introducing cracks or defects. These defects make it difficult to maintain consistent, predictable gel morphologies on micron length scales and longer.

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As a means for embedding cells within defined micron-scale particles, microgels have received significant interest within the past several years for a range of applications, including stem cell encapsulation and delivery^{16, 17} and tissue engineering.^{16–23} For a recent review, see Elbert.²⁴ Recent microgel strategies have been introduced whereby different cell types can be encapsulated and then assembled together in a modular fashion to produce 3D engineered tissue constructs.^{18, 21, 23} Cell-encapsulating microgels have been produced using a variety of approaches, including thermally induced gelation of collagen/agarose composites within emulsions,^{16, 17} collagen gelation within molds,¹⁸ photoinitiated cross-linking of PEGs under photomasks,^{21, 25} stop-flow lithography,²⁶ and peptide assembly within microfluidics.²⁷ In the present work, a strategy was developed for producing microgels from self-assembled peptides, enabling cell encapsulation and handling without the heterogeneous gel fracture that has previously limited self-assembled peptide materials.

We have previously investigated scaffolds based on short β -sheet fibrillizing peptides for constructing multi-peptide, chemically defined analogs of extracellular matrices (ECMs), primarily based on the short peptide Q11 (Ac-QQKFQFQFEQQ-Am).^{14, 15, 28, 29} In comparison with synthetic polymers or materials based on naturally sourced whole proteins, self-assembling biomaterials based on small chemically defined molecules offer routes for precisely integrating several different molecular features into insoluble matrices, including cell-binding peptides^{8, 14, 15} and chemoselective cross-linking groups.²⁹ Self-assembly processes also tend to have favorable biocompatibility; in most cases they do not depend on solvents, temperature changes, or covalent chemistry. Rather, the assembly of these materials is sensitive to the ionic strength of the solution,³⁰ which has been exploited in the present work to fabricate microgels. Ionic strength modulation has been employed routinely to gel solutions of several different types of β -sheet fibrillizing peptides, and has been achieved by pipetting the peptide solutions into buffers,⁷ by overlaying peptide solutions with buffers,^{14, 29} by delivering the peptides to tissues,⁹ or by releasing salts into the peptide solutions from light- or temperature-sensitive liposomes.³¹ Here, ionic strength changes were combined with emulsion processing to produce self-assembled peptide microgels (Figure 1), which could subsequently be used to encapsulate cells or to create multi-peptide gels with domains of one peptide type embedded in a second type.

RESULTS

Triggered self-assembly in emulsions

Microgels were produced using the conditionally assembling peptide Q11,^{15, 28, 29} combined with emulsion processing (Figure 1, Figure 2), making use of the sensitivity of Q11's assembly to ionic strength, a property it shares with other fibrillizing peptides previously reported.^{7, 32} When solutions of Q11 were added to USP mineral oil and mixed, the aqueous peptide could be gelled by adding PBS to the homogenate. Spherical peptide microgels formed for all blade speeds tested (Figure 2a–e), with the smallest microgels being formed when a post-gelation shear step was employed (histograms of microgel size shown in Figure 3). Owing to the amphiphilic character of Q11, it was possible that the oil/water interface could have disrupted assembly of the peptide, so the morphology of the microgels' constituent fibrils was characterized with TEM. It was found that emulsion-processed peptide fibrils were morphologically similar to previously reported fibrils of Q11, having diameters of around 10nm, exhibiting significant lateral aggregation, and being relatively unbranched (Figure 2f). Peptide yield ranged from 14–25% depending on processing conditions, with the majority of the loss occurring during extraction owing to the formation of a peptide-rich interphase. This aspect could be improved in the future using modified techniques such as gradient centrifugation.

Once formed, the microgels were stable enough to be transferred between containers by pipetting, allowing the construction of multi-peptide materials with micron-scale domains of specified peptide formulations. This was demonstrated by producing microgels composed of 30 mM Q11, staining them with Congo red, and suspending the stained microgels in a second solution of Q11 labeled with a green NBD fluorophore (Figure 2g). Overlaying this composite material with PBS induced gelation in the NBD-labeled peptide, forming a matrix around the embedded microgels that could be visualized using confocal microscopy. Owing to the mild conditions under which these materials were formed, that is only the sequential application of buffer constituents rather than covalent polymerization processes or thermally induced gelation, this approach may hold promise as a simple method for constructing 3D cell culture matrices having discrete domains of different peptide compositions.

The microgel fabrication process was robust, with the size distribution of the formed microgels being more dependent on the speed and type of the mixer than on the volume or concentration of the aqueous phase (Figure 3). For peptide concentrations between 10–50 mM, the histograms of size distribution were largely overlapping (Figure 4a). This was also generally true for volumes of aqueous phase between 30–600 μ L (Figure 4b), though a volume of 150 μ L produced the most uniformly sized particles. The most spherical aspect ratios were produced by the paddle-type stirrer and by a homogenizer speed of 9,000 rpm (Figure 4c), and for all speeds the most prevalent aspect ratios were between 1–1.4. Interestingly, employment of the post-gelation shear step did not significantly alter the aspect ratio of the microgels. Low volumes of aqueous phase also produced the most spherical microgels, though the most prevalent aspect ratio for all volumes tested was between 1–1.3 (Figure 4d). These results indicated that the process was relatively scalable and amenable to a range of different peptide concentrations and volumes that could be chosen based on the needs of a particular cell encapsulation application. In contrast, a degree of control could be achieved over the average diameter of the microgels by varying the type and speed of the homogenizer blade (Figure 3). The largest microgels were generated by the paddle-type stirrer, which produced a broad range of microgel sizes between 5–120 μ m. Microgels produced with the homogenizer showed tighter size distributions, mainly between 5–50 μ m, with a tail of larger-diameter microgels particularly evident for slower homogenizer speeds. This tail could be eliminated using the fastest rotation speeds. At 12,000–21,000 rpm, microgel sizes were confined between 5–40 μ m. The smallest microgels could be generated by employing the post-gelation shear step, producing microgel sizes below 15 μ m (Figure 3).

Cell encapsulation

When cells were mixed with the precursor peptide solution, batches of microgels were produced containing viable encapsulated cells. Microgels with encapsulated NIH 3T3 fibroblasts were generally spherical (Figure 5a–c). Viability was about 80% immediately after processing, a level that was maintained for at least 3 days in culture, and that was independent of the blade type used (Figure 5d). We found that the primary source of cell death was transient acidity of the peptide precursor solution, as cells processed in initially buffered solutions showed increased viability. Few unencapsulated cells were observed, as evidenced by a negligible reduction in MTS signal when cell-laden microgels were plated onto cell culture microplates and then transferred to fresh plates after allowing sufficient time for unencapsulated cells to attach to the plate (4 hours, Figure 6a). This finding also indicated that the cells could be transferred from one container to another without significant loss. The MTS signal from acellular microgels was similar to medium controls (not shown), indicating minimal interference by the gels in the MTS signals. Initial yields of cells were about 10%, similar to the yield of peptide. During culture, an increase in cell density was qualitatively observed during the live/dead analysis (Figure 5a–c), a finding that was

quantified by performing MTS assays on cultured microgels over four days (Figure 6b). In microgels, 3T3 cells exhibited significant growth, with an apparent doubling time of 6 h over the first day of culture (Figure 6b). This rate was much greater than the 23 h doubling time observed for the same cells seeded directly onto microplates (not shown), a value more typical for this cell type in 2D culture.³³ It is possible that the change in the MTS measurements over the first day of culture reflected both a recovery of the cells from the emulsion processing as well as actual growth of the cells, and on days 2 and 4, this apparent growth rate decreased, most likely indicating crowding in the microgel and confluence being reached. Overall, the rapid initial cell growth along with the consistently good viability levels was a first indication that the microgels formed acceptable microenvironments for 3T3 cell growth. Morphologically, cells in the microgels adopted a more rounded phenotype compared to cells cultured on plastic dishes, as is commonly observed for 3D cultures.

Mouse pluripotent C3H10T $\frac{1}{2}$ stem cells were also encapsulated within microgels. Although the survival of the cells immediately following microgel fabrication was slightly lower than for 3T3 cells, about 60%, viability improved over several days in culture when 6 mM RGD-Q11 was included within the microgels, to 83% viability after 3 days (Figure 6c). In contrast, microgels containing scrambled RDG-Q11 in the place of RGD-Q11 did not exhibit this improvement, suggesting that the RGD-Q11 specifically enhanced cell growth. Although there was significant growth in these cultures, the microgels did not change in size during this time period (Figure 6d), suggesting that significant space was available within the microgels to allow proliferation. Previously, we reported Q11-based gels in which the amounts of multiple different short peptide ligands were systematically adjusted using Design of Experiments approaches.¹⁴ The present results indicating the responsiveness of C3H10T $\frac{1}{2}$ cells to RGD-containing microgels make it likely that such multifactorial approaches would also be applicable for this cell type in 3D microgel formats.

DISCUSSION

We have developed a reproducible method for creating microspheres from self-assembled peptide gels. The approach is based on the salt-sensitivity of the peptide Q11, which is initially soluble in pure water but forms entangled networks of fibrils when exposed to neutral buffers and millimolar concentrations of monovalent or divalent salts.^{15, 28, 29} In the work reported here, the gelation-inducing reagent was Dulbecco's PBS, enabling microgel formation within emulsions using conditions that are physiologically benign. The microgels were durable and could be collected by centrifugation and resuspension in buffer or cell culture media, as demonstrated by the size characterization experiments, composite matrix construction, and cell encapsulation experiments. This approach should be generally applicable to the several self-assembling systems that have been reported to date that are sensitive to ionic strength. These include other β -sheet fibrillizing peptides,^{1, 7, 32} β -hairpins,^{12, 34} peptide amphiphiles,³⁵ and short aromatic peptide derivatives.⁵

Self-assembling peptides have previously been employed to provide synthetic cell culture matrices with precisely defined compositions, including our earlier work where multiple ligands within multi-peptide assemblies were systematically adjusted using factorial experimental designs and response surface modeling, in an effort to precisely optimize the matrices for rapid endothelialization.¹⁴ The present work extends the type of definition possible with these materials from molecular length scales to micron scales. For example, we illustrate here that gels containing micron-scale domains of different peptide formulations can be constructed by sequentially triggering self-assembly, first in microgels and then in a matrix surrounding those microgels (Figure 2g). One could envision extending these processes, for example by entrapping more than one different type of microgel within the matrix, by embedding different populations of cell types, or by designing serial

emulsification processes where multilayered structures could be produced. Given that the system's assembly is only dependent on the application of salt-containing buffers to unassembled peptide, complex structures with multiple cell types and multiple regions of different peptide types can be readily envisioned.

One shortcoming of the strategy described here is that it does not feature as tight of control over microgel size compared to other processes such as axisymmetric flow strategies or aqueous free-radical precipitation polymerization,^{27, 36} which can produce more monodisperse populations. It may be possible to combine Q11 and its derivatives with axisymmetric flow strategies, as has been recently described for the RADA-16 peptide, which was gelled by the migration of powdered salts from the oil phase to the peptide-containing water phase.²⁷ The trade-off with serially-produced microgels such as these, however, is that such serial processing of microgels may not be as scalable as batch processing.

In terms of tissue engineering and cell encapsulation technologies, the microgel approach reported here is advantageous because it serves to define the materials on the scale of tens-to-hundreds of microns. Because bulk self-assembled peptide gels are prone to fracture, they are difficult to handle in culture or upon in vivo delivery without introducing cracks and heterogeneities. These heterogeneities in turn would be expected to dominate transport of media or physiological fluids throughout and within the materials. In contrast, encapsulating cells in peptide microgels allows the experimenter to transfer them from one container to another without fracturing them, as evidenced by the transfer of the cell-laden gels to culture dishes at the end of the fabrication procedure and the construction of peptide gels with embedded microgels of different peptide formulations (Figure 2, Figure 6).

EXPERIMENTAL

Peptide synthesis

Peptides Q11 (Ac-QQKFQFQFEQQ-Am), RGD-Q11 (Ac-GGRGDSGGGQQKFQFQFEQQ-Am), RDG-Q11 (Ac-GGRDGS GGGQQKFQFQFEQQ), and fluorescent NBD-RGD-Q11 (nitrobenzoxadiazole-GGRGDSGGGQQKFQFQFEQQ-Am) were synthesized on a CS Bio 136 peptide synthesizer as previously reported using standard Fmoc protocols.^{15, 29} NBD was conjugated to the N-terminus using NBD-succinimidyl ester (Invitrogen, S-1167). Peptide identity was determined using MALDI mass spectrometry; Q11 [M+H]⁺ *m/z* calc'd: 1527.7, found: 1527.4; RGD-Q11 [M+H]⁺ *m/z* calc'd: 2228.3, found: 2228.7; NBD-RGD-Q11 [M+H]⁺ *m/z* calc'd: 2462.6, found: 2462.6; RDG-Q11 [M+H]⁺ *m/z* calc'd: 2228.3, found: 2228.3.

Microgel fabrication

Microgels were fabricated using the scheme shown in Figure 1. Peptides were first mixed in deionized water in concentrations between 10–50 mM, then incubated for 3 days to allow initial assembly. Separately, USP/NF mineral oil was mixed with either a blade-type homogenizer (Brinkman Polytron PT 3000) or a paddle-type stirrer (IKA RW 11). For the homogenizer, 5 mL oil was mixed in a 10 mL beaker, and for the stirrer, 30 mL oil was mixed in a 50 mL beaker. Care was taken not to introduce air bubbles into the mixed oil. Blade speeds were varied between 1,000–21,000 rpm for the homogenizer and 1500–2000 rpm for the stirrer. To the continuously stirred mineral oil was added 30–600 μ L of peptide solution, and stirring was continued for 1 min to form uniform emulsions. To initiate gelation of the peptides in the aqueous phase, the blade speed was slowed to 1,000 rpm, and Dulbecco's phosphate buffered saline (PBS) was immediately added to the stirred homogenate (1 mL in the case of the homogenizer, 5 mL in the case of the stirrer). The homogenate was then mixed for 15 s at the reduced speed of 1,000 rpm to allow fusion of

peptide and PBS droplets, after which stirring was halted. Some preparations were additionally subjected to a post-gelation shear step, in which the homogenizer speed was increased to 6,000 rpm for 15 minutes. Finally, the formed microgels were isolated by transferring the homogenate into excess PBS (5 mL in the case of microgels produced with the homogenizer; 10 mL in the case of microgels produced with the stirrer), shaking, and centrifugation at 240 rcf for 3 min. In this step, the microgels partitioned within the PBS phase, and the oil was discarded.

Morphological Characterization

To create fluorescent microgels, 50 μ M NBD-RGD-Q11 was added to the peptide solutions before emulsification. Alternatively, formed microgels were stained with 50 μ M Congo red after fabrication. The microgels' sizes and shape factors were assessed with respect to several process variables, including peptide concentration (10–50 mM), volume of the aqueous phase (30–600 μ L), blade type (paddle-type or rotor-stator homogenizer), rotor speed (1,000–18,000 rpm for homogenizer), and inclusion of the post-gelation shear step. Each factor was in turn varied independently, while holding all other variables at their mid-point values (30 mM for peptide concentration, 150 μ L for volume of the aqueous phase, and 3,000 rpm for homogenizer speed). Solutions containing processed microgels were mounted on 96-well polystyrene plates, which were lightly centrifuged before observation to ensure that the microgels were all located on the bottom of the plate, on the same focal plane. Fluorescent images were acquired with a Zeiss Axioskop epifluorescence microscope. Microgel counts and dimensions were then analyzed with Image J software. For each different fabrication condition, 200–1000 microgels were analyzed. Diameters were calculated by measuring the area of each microgel and assuming a spherical particle. Aspect ratios were calculated by fitting an ellipse over each particle using Image J software and dividing the long axis by the short axis. A “polydispersity” for particle diameter was calculated in a manner analogous to the calculation of polydispersity for polymers. That is, a number average was calculated as the arithmetic mean of the diameters, a weighted average was calculated, and the polydispersity index was the ratio between the two.

$$\begin{aligned} \text{number average} &= \frac{\sum D_i \cdot N_i}{\sum N_i} \\ \text{weight average} &= \frac{\sum D_i^2 \cdot N_i}{\sum D_i \cdot N_i} \\ \text{Polydispersity Index (P.I.)} &= \frac{\text{weight average}}{\text{number average}} \end{aligned} \quad \text{Eq. 1}$$

In which D_i represents the diameter of the i th microgel, and N_i represents the number of microgels having diameter D_i .

To calculate the yield of peptide that was present after the fabrication process, microgels were synthesized at the lowest and highest values for each varied parameter, plus at the midpoints. Processed microgels were collected, and buffer salts were removed by serially washing them three times with water, removing the water after each wash by light centrifugation and decanting. Microgels were then lyophilized and weighed.

Synthesis and characterization of multi-peptide gels with microscale domains

Gels containing microscale domains of different peptide formulations were visualized using Congo red and NBD. For Congo red staining, microgels were incubated in an aqueous solution of 50 μ M Congo red overnight, followed by serial washes with deionized water to remove unbound stain and buffer salts. Stained microgels were suspended in a solution of 30 mM aqueous Q11 (not assembled) containing 50 μ M NBD-RGD-Q11, which was then gelled by overlaying it with PBS, thus producing 30 mM NBD-labeled RGD-Q11 gels with

embedded domains of 30 mM Congo red-stained Q11. Gels were imaged using a Zeiss LSM 510 confocal microscope.

Characterization of fibril morphology

To determine whether the emulsion processing affected the fibrillization of Q11-based peptides, individual fibrils were isolated from microgels for TEM analysis. This was accomplished by taking formed microgels, diluting them in excess PBS, and homogenizing them at 1500 rpm. The disrupted microgels were then diluted 1:100 in water, applied to 400 mesh copper TEM grids with carbon support films, and stained with uranyl acetate. Fibrils extracted and prepared in this way were imaged with a Tencai F30 scanning transmission electron microscope.

Cell encapsulation and viability

NIH 3T3 cells were cultured in DMEM with 10% FBS and 2mM L-glutamine. Mouse embryonic pluripotent C3H10T $\frac{1}{2}$ stem cells were cultured in basal medium Eagle (BME) containing 10% FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, and 1X non-essential amino acids (Cellgro cat# 25-025-cl). For encapsulation, cells were trypsinized and suspended in 10% sucrose containing 180 μ g/mL penicillin, 300 μ g/mL streptomycin, and 0.75 μ g/mL amphotericin B, at a density of 1.2×10^7 cells/mL. This cell suspension was then mixed 1:2 with peptide solutions containing either 45 mM Q11, 36 mM Q11/9 mM RGD-Q11, or 36 mM Q11/9 mM RDG-Q11, forming a working cell suspension containing 4×10^6 cells/mL and 30 mM total peptide. This solution was then immediately processed into microgels as described above using an emulsification time of 5 seconds. Microgels collected in PBS were immediately resuspended in culture media. To assess viability, cells were stained with calcein-AM, a live-cell marker, and ethidium homodimer-1, a dead-cell marker (Invitrogen L3224). Cell viabilities were quantified at 2 hours, 1 day, 2 days, and 3 days after microgel fabrication. To measure encapsulation efficiency and cell growth, an MTS assay was used (Promega cat# G3582), in a similar manner as previously reported.^{14, 15, 29} This assay measures metabolic activity and is commonly employed as a correlate of proliferation, though the magnitude of the MTS signal is also affected by apoptotic events and cell survival, in addition to proliferation. Cell-laden microgels were cultured in 96-well suspension culture plates (Greiner cat# 655185), with the exception of the experiment specifically designed to measure the amount of unencapsulated cells (Figure 6a), for which cell-adhesive culture plates were utilized (BD cat# 353072). Acellular microgels and cell culture media were used as negative controls. For measuring encapsulation efficiency, 7.5×10^5 cells were encapsulated within 30 mM Q11 microgels at a density of 4×10^6 cells/mL in the precursor cell/peptide suspension. After processing, the cell-laden microgels were divided evenly into 20 wells of 96-well plates. For comparison, the same number of cells was seeded directly into 20 wells of 96-well microplates (BD cat# 353072) without encapsulation. Acellular microgels incubated in media were used as negative controls. Immediately before the MTS assay, microgels were transferred into fresh 96-well plates to minimize background. Viability measurements and MTS assays were compared using ANOVA with Tukey post-hoc testing, with sample sizes of at least 4 independent wells. The cell growth experiments were conducted in duplicate, with similar results.

CONCLUSION

Spherical microgels were fabricated using peptide self-assembly and water-in-oil emulsification. The size distributions of the microgels were adjustable within a range of several microns to several hundred microns, by changing fabrication process parameters, most notably the rotor speed and the employment of a post-gelation shear step. Other processing parameters, including volume and peptide concentration, did not significantly

influence peptide size distribution. Composites were created consisting of microgels embedded within a matrix of a second peptide formulation using only buffer-triggered assembly, providing routes for multi-peptide matrices with defined micron-scale domains. The microgel fabrication process was appropriate for cell encapsulation, and NIH 3T3 and C3H10T½ murine embryonic pluripotent stem cells were encapsulated with good viability. The strategy described offers a simple route for producing spherical gels of self-assembled peptides, using only buffer addition as a trigger, making it potentially useful for constructing cell/matrix composites with micron-scale dimensional control.

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Figure 1. Schematic for microgel fabrication. A solution of Q11 peptides with or without additional cells was added to USP mineral oil and emulsified (1). After formation of a water-in-oil emulsion, a small volume of PBS was added, gelling the peptide in the aqueous phase (2). Microgels were then extracted in excess PBS and collected by centrifugation (3).

Figure 2.

Fluorescent NBD-containing microgels produced with various processing conditions (a–e). Microgels were produced with a paddle-type stirrer (a), a homogenizer at different rotation speeds (b–d): 1,000 rpm (b), 3,000 rpm (c), and 18,000 rpm (d), and with an additional post-gelation shear step (e). Scale bar in a–e, 50 μm. Emulsion processing did not disrupt the fibrillar architecture of self-assembled Q11 peptide, as observed with TEM and negative staining (f, scale bar 100 nm). Microgels of one peptide type could be suspended within a matrix of a second self-assembling peptide, as illustrated by laser scanning confocal microscopy (g) of Congo red-stained microgels of 30 mM Q11 (red) embedded within a matrix of 30 mM Q11 labeled with 50 μM NBD-RGD-Q11 (green). x–y horizontal plane (top) and x–z vertical plane (bottom) of different locations in the gel. Scale bar in g, 20 μm.

Figure 3.

Size distributions of microgels with varying process conditions. Microgel size distributions were influenced by the type and speed of the mixing blade employed. Stirrer at 2,000 rpm (a). Homogenizer at 3,000 rpm; 12,000 rpm; and 21,000 rpm (b–d, respectively). With post-gelation shearing step (e). Polydispersity indexes calculated by Eq. 1 (see Experimental section).

Figure 4.

Influence of processing conditions on microgel diameter (a, b) and aspect ratio (c, d). Peptide concentration (a) and volume of the aqueous phase (b) had only small influences on microgel size. Aspect ratios were moderately influenced by blade type and speed (c), as well as the volume of the aqueous phase (d).

Figure 5.

The viability of NIH 3T3 cells encapsulated in 30 mM Q11 microgels was quantified with calcein/ethidium homodimer staining. The assay was conducted 2 hours after encapsulation (a), 1 day (b), 2 days (c) and 3 days after encapsulation. After encapsulation, high cell viability was maintained for at least three days, for microgels processed with the homogenizer (●) and the paddle-type stirrer (○) (d, n = 10 microgels). Scale bar in (a) = 100 μm, same magnification in a–c.

Figure 6.

Yield of NIH 3T3 fibroblast cell encapsulation and growth over time in 30 mM Q11 microgels, as quantified by MTS assays. Microgels seeded onto microplates and those that were transferred to fresh microplates after initial cell attachment showed similar cell numbers, indicating minimal presence of unencapsulated cells (a). Although initial cell yield was low, 10% of feed, the cell number recovered by 24h to around 50% of feed, presumably through cell proliferation. Continued growth of encapsulated NIH 3T3 cells was observed with MTS (b). Viability of C3H10T $\frac{1}{2}$ cells in microgels containing 30 mM peptide (● 24 mM Q11 + 6 mM RGD-Q11; ▽ 24 mM Q11 + 6 mM RDG-Q11) (c). * $p < 0.05$ by ANOVA with Tukey post-hoc testing, $n = 4$ independent wells (a–b) or $n = 10$ microgels (c). The diameters of the microgels whose viability is shown in (c) did not change over time in culture (d, not significant by ANOVA with Tukey post-hoc testing).