tronic rearrangement of the fulvalene ligand (Scheme I). Much to our surprise, the same reaction carried out in the presence of 2 equiv of $Na^+PF_6^-$ gave a major product which did not show CO IR bands. The stable red complex **3**[8] obtained in 35% yield has a rare[6f] monometallic fulvalene structure. The elemental analysis shows the presence of the Na^+ and PF_6^- counterions.[8] The cationic Fe^{II} d^6 zwitterionic state is confirmed not only by the NMR and Mössbauer data[8] but also by the cyclic voltammogram, characteristic of the $[Fe^{II}Cp(arene)]^+$ series (a reversible 1-electron wave at −1.32 V vs SCE and a chemically irreversible 1-electron wave at −2.23 V (i_a/i_c = 0 at 20 °C and 0.2 at −50 °C); irreversible oxidation of Cp^- at +0.81 V, 0.4 V s^{-1}, DMF, 0.1 M nBu_4NBF_4, Pt). Thus the $Fe(CO)_2$ fragment was lost from the proposed intermediate A in Scheme I. Compare with the reaction of the mononuclear 19-electron complex $[Fe^ICp(C_6H_6)]$[6c,d] with CO in THF which was reported to give $[FeCp(CO)_2]_2$ whether or not $Na^+PF_6^-$ was present.[9] The change of reaction of **1** in the presence of $Na^+PF_6^-$ is best taken into account by a double ion exchange[5] between the two ion pairs B and $Na^+PF_6^-$ (Scheme I). In B, there is the possibility of stabilization of the zwitterion by intramolecular ion pairing allowed by the free rotation about the Cp–Cp bond (ET is roughly isoergonic[4b,6] and reversible), but this $Fe^+\cdots Fe^-$ interaction must be dislocated by $Na^+PF_6^-$, which drives the formation of **3**.

The reaction of **1** with PMe_3 in THF at −20 °C in the absence of $Na^+PF_6^-$ follows a course similar to that with CO, giving the thermally unstable diamagnetic orange-red complex **4** (Scheme II). The latter was characterized inter alia by the symmetrical fulvalene ^1H and ^{31}P NMR pattern[10] and the mass spectrum, giving a minor peak at $(M = PMe_3)^+$ and major peaks at $(PMe_3)^+$, $(M - 4PMe_3)^+$, and $(FeFv)_n^+$, n = 2 and 3 (biferrocenylene and triferrocenylene, respectively).

The same reaction in the presence of 2 equiv of $Na^+PF_6^-$ gives the stable, orange, new diamagnetic d^6 dication **5** (50%)[11] and the known complex $[Fe(PMe_3)_4]$[9,12] (30%), the formation of which is driven by the decomposition of 1^-Na^+ as in the case of the monoiron chemistry.[9] The formation of **5** (in the maximum disproportionation yield) provides a very efficient entry from sandwich into new piano stool diiron fulvalene chemistry.

Thus from *intramolecular* ET with CO, the $Na^+PF_6^-$-induced ET becomes *intermolecular* with PMe_3. Each time the salt effect is quantitative as in monoiron chemistry[9,13,14] using $Na^+PF_6^-$, a very efficient although common salt. This way to direct and control ET using a simple Na^+ salt brings about a very versatile synthetic tool in molecular chemistry. As an illustration, the electronic communication betwen two metal centers mediated across a delocalized hydrocarbon ligand was induced and modulated.

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(7) (a) $[Fe_2(\mu_2\text{-Fv})(CO)_6]$ (**2**): ^1H NMR ($CDCl_3$, δ ppm) 5.34, 5.05 (2t, C_5H_4, 2 × 4 H); ^{13}C NMR ($CDCl_3$) 218.63 (*C*O), 88.62, 86.53, 76.03 (C_5H_4); IR (CH_2Cl_2) ν_{CO} 2000, 1940 cm^{-1}; slightly thermally unstable at 20 °C. (b) Butenschön recently reported $[Fe_2(\mu_2\text{-Fv})(CO)_4]$, which differs from **2** inter alia by its stability and NMR and IR: Bister, H. J.; Butenschön, H. *Synlett* **1992**, 22.

(8) $[Fe_2(\eta^5\text{-Fv})(C_6H_6)(NaPF_6)]$ (**3**): ^1H NMR (CD_3COCD_3, δ ppm) 6.10 (s, C_6H_6, 6 H), 5.24, 5.08, 4.70, 4.47 (4t, C_5H_4, 4 × 2 H); ^{13}C NMR (CD_3COCD_3) 89.6 (C_6H_6), 96.31, 78.23, 76.83, 73.61, 72.44, 69.31 ($C_5H_4C_5H_4$); Mössbauer (mm/s vs Fe, 293 K) I.S. 0.52, Q.S. 1.61; CV (DMF, 0.1 M nBu_4NBF_4, Pt, 20 °C, 0.4 V/s) $E°$ (V vs SCE) − 1.32 V (i_a/i_c = 0.8), ΔE_p = 90 mV, E_{pc} = −2.23 V (irrev), E_{pa} = +0.81 V (i_c/i_a = 0), (−50 °C) $E°$ (V vs SCE) −1.35 V (i_a/i_c = 1), 2.19 V (i_a/i_c = 0.2), ΔE_p = 170 mV, +0.84 (irrev). Anal. Calcd for $C_{16}H_{14}FeNaPF_6$: C, 44.68; H, 3.28. Found: C, 44.87; H, 3.30. The modest yield of **3** is due to the instability of the diradical **1** above −10 °C; 10% of the oxidation product $[Fe^+(C_6H_6)(\mu_2\text{-Fv})(CO)_2]_2\text{-}(PF_6^-)_2$ was also found.

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(10) (a) $[Fe_2(\mu_2\text{-Fv})(PMe_3)_4]$, (**4**): ^1H NMR ($C_6D_5CD_3$, δ ppm) 3.652, 3.345 (2t, C_5H_4, 2 × 4 H); 1.458–0.987 (2m, CH_3, 36 H); ^{31}P NMR ($C_6D_5CD_3$, δ vs 85% H_3PO_4) 26.70. (b) Butenschön's complex[7b] is the "carbonyl equivalent" of **4**.

(11) $[Fe_2(\mu_2\text{-Fv})(PMe_3)_6](PF_6^-)_2$ (**5**): ^1H NMR (CD_3CN, δ ppm) 5.04, 4.63 (2m, $C_5H_4C_5H_4$, 8 H), 1.55 (m, Me, 54 H); ^{31}P NMR (CD_3COCD_3) 22.4; ^{13}C NMR (CD_3COCD_3) 88.23, 82.9, 78.35 ($C_5H_4C_5H_4$), 22.23 (m, $P(CH_3)_3$); Mössbauer (mm/s vs Fe, 293 K) I.S. 0.320, Q.S. 1.762; CV (DMF 0.1 M nBu_4NBF_4, Pt, −30 °C, 0.4 V/s) E (V vs SCE) − 1.65 (i_a/i_c = 0), + 0.70 (i_c/i_a = 1), ΔE_p = 70 mV, + 1.08 (i_c/i_a = 0.9; quasi-reversible). Anal. Calcd for $C_{28}H_{62}Fe_2P_8F_{12}$: C, 34.07; H, 6.28. Found: C, 34.50; H, 5.74. (b) The salt-induced ET reaction (Scheme II) is the only route to **6** (for instance, photolysis of $1^{2+}(PF_6^-)_2$ in CH_3CN in the presence of excess PMe_3 could not yield **6**).

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Rapid Photopolymerization of Immunoprotective Gels in Contact with Cells and Tissue

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Microencapsulation and cell transplantation technology hold promise in many areas of medicine and biotechnology, such as the treatment of diabetes[1-4] and the evaluation of candidate antiviral[5] and antitumor[6] drugs. Successful microencapsulation of cells requires that the cells survive encapsulation and retain their normal function, that the membrane be stable under physiological conditions over several years, and that it be permselective with a molecular weight cutoff in the range 50 000–100 000 Da so as to be immunoprotective. It is also important that the microcapsules be biocompatible so as to resist a fibrous reaction by the host, as is seen in some currently investigated microcapsules,[2,7] which can greatly reduce oxygen and nutrient diffusion to the transplanted cells.

The polymerization of materials in intimate contact with cells or tissue without loss of viability is quite difficult. Polymerization of acrylamide monomer upon microspherical scaffolds of agarose-containing cells has been performed successfully,[8] but this can generate excessive local heating and cytotoxicity if attempted directly on tissue.[9] Here we report the synthesis of stable, biocompatible gels with permselectivity appropriate for immunoprotection via rapid photopolymerization of water-soluble poly(ethylene glycol)-based macromers in direct contact with cells and tissue without cytotoxicity. The particular polymerization scheme chosen permitted gelation in the presence of dissolved oxygen, which is generally important in maintaining cell viability.

The use of poly(ethylene glycol) (PEG) to obtain biocompatibility by reducing protein adsorption, cell adhesion, and fibrous encapsulation of materials is well established.[10-13] PEG diacrylates

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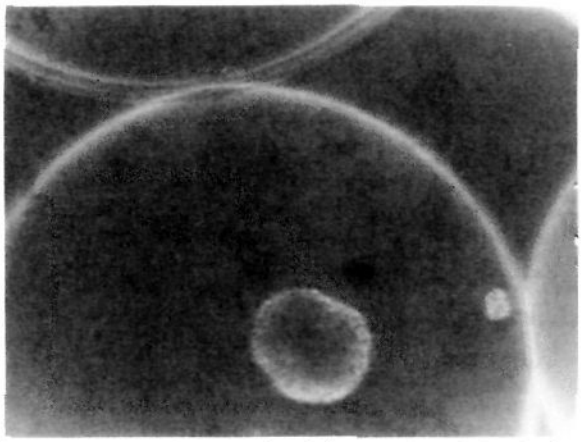
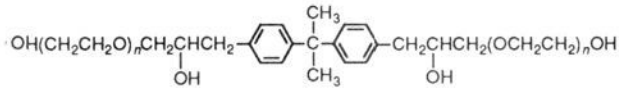


Figure 1. Microspheres produced by the laser-induced photopolymerization of 18.5K MA; magnification 50X.

and multiacrylates of various molecular weights were synthesized by reaction of PEG with acryloyl chloride (4-fold excess based on PEG hydroxyl end groups), using triethylamine (equimolar with acryloyl chloride) as a proton acceptor in a dry benzene reflux under a dry nitrogen atmosphere. The resulting product was filtered and precipitated with hexane. It was redissolved in benzene and reprecipitated in an excess of hexane, filtered, and dried under vacuum at 60 °C. Tetrahydroxy PEG (MW 18 500) with the structure shown here was purchased from Polysciences and used for the multiacrylate synthesis; small amounts of higher molecular weight polymers are present in the tetrahydroxy preparation, but the predominant component is the tetrahydroxy PEG as shown. Other PEGs (MW 400–35 000) were α,ω-dihydroxy and were obtained from Aldrich and Polysciences.



A solution (23% w/w in 10 mM HEPES buffered saline) of the PEG multiacrylate (18.5K MA) was mixed with a cell suspension along with ethyl eosin (0.5 mM) and triethanolamine (100 mM) as the photosensitizer/electron donor initiating system and 1 μL/mL of *N*-vinylpyrrolidinone. The rate of polymerization was followed by measuring the length of a spike produced by the gelation of macromer within a square cuvette following penetration of an argon ion laser beam (500 mW, beam diameter 3 mm, emission max = 514 nm). The mass of gel produced was observed to be an exponential function of irradiation time. Using this information it was estimated that a sphere 500 μm in diameter could be polymerized in about 100 ms at this illumination intensity (6 W/cm^2). A coextrusion apparatus was used to form microspheres 0.5–0.8 mm in diameter by extruding the liquid macromer into droplets and subsequently gelling them by exposure to laser light.[14] The viability of the encapsulated cells was measured by trypan blue exclusion assay (Sigma). Human foreskin fibroblasts (HFF), Chinese hamster ovary cells (CHO-K1), and β cell insuloma cells (RiN5F)[15] were found to be viable (more than 95%) after encapsulation; the viability of control cells without encapsulation was also 95%. Figure 1 shows microspheres, containing rat islets of Langerhans, produced by this method. The laser light is not absorbed by cells in the absence of an exogenous, cell-binding chromophore.[16] There is no significant heat of polymerization

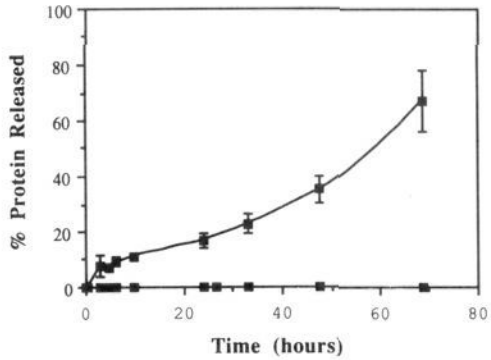


Figure 2. Release profiles of BSA (□), IgG (⊡), and Fg (◆) from hydrogels made from the 18.5K MA precursor. No significant release of IgG and Fg is evident. Each data point is the mean of two measurements.

due to the nature, size, and dilution of the macromers used. These polymerizations can proceed extremely rapidly in oxygen-containing aqueous environments at physiological pH. Rat islets of Langerhans were encapsulated and examined ultrastructurally by transmission electron microscopy; ultrastructure was different from control only at the outermost single cell layer, where the β cells showed fewer secretory granules than untreated control islets. The absence of tissue necrosis on intimate contact with polymerizing 18.5K MA gel was observed on the liver of the rat, a particularly sensitive tissue. The liver was excised 10 days postoperatively and the epithelium appeared normal, with no evidence of inflammation or necrosis on the liver surface. We have also investigated similar materials in vivo in a rabbit uterine horn model for postoperative adhesion prevention with similar results.[17]

Microspherical gels made from the 18.5K MA precursor were stable for over 1 year in phosphate-buffered saline. Gels made from the 18.5K MA precursor contained 91.4 ± 0.9% water, and the fraction of macromer undergoing gelation was seen to be 80.3 ± 0.9%. Disc-shaped gels made from this precursor were implanted subcutaneously in 4 week old rats and were monitored for calcification and mechanical deterioration. These gels did not calcify or lose tensile strength significantly when compared to gels of the same composition that had not been implanted (control) over an 8-week implantation period.[18]

Gels with the proper formulation were found to be capable of being immunoprotective as indicated by protein diffusion.[19] Figure 2 shows the diffusion profiles of proteins through a PEG 10 000 diacrylate gel; this gel allowed the slow diffusion of bovine serum albumin (BSA) (MW 67 000), but was impermeable to immunoglobulin G (IgG) (MW approximately 150 000) and fibrinogen (Fg) (MW approximately 350 000).

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Supplementary Material Available: A figure showing details of the coextrusion apparatus for fabrication of microspheres along with a description of the apparatus and a table on the change in tensile strength and increase in calcium content of 18.5K MA gels on implantation in juvenile rats (4 pages). Ordering information is given on any current masthead page.

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(19) Ten milligrams of bovine serum albumin, human immunoglobulins, or human fibrinogen were dissolved in 2 mL of 23% PEG 10 000 diacrylate macromer solution in PBS. The entire solution was gelled using an argon ion laser. The release of these proteins from the gel into 10 mL of PBS was monitored spectrophotometrically over time using a protein assay reagent (BIORAD No. 5000006).