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Radiopaque Alginate Microcapsules for X-ray Visualization and Immunoprotection of Cellular Therapeutics

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Abstract: Alginate-poly-L-lysine-alginate (APA) microcapsules have been explored as vehicles for therapeutic drug and cell delivery. The permselectivity of these capsules provides a unique means of controlled drug release and immunoisolation of encapsulated cells. Immunoisolation is especially attractive as it abrogates the need for chronic immunosuppressive therapy and opens up the possibility for the delivery of numerous cell sources including xenogeneic grafts. APA microcapsules containing cellular therapeutics have proven effective in the short-term treatment of a wide range of diseases requiring enzyme or endocrine replacement therapy, including type I diabetes. If these microcapsules could be noninvasively monitored with X-ray imaging modalities (i.e., fluoroscopy, CT, and digital subtraction angiography), questions such as the ideal transplantation site, the best means of delivery, and the long-term survival of grafts could be better addressed. We have developed two novel alginate-based radiopaque microcapsule formulations containing either barium sulfate (Ba X-Caps) or bismuth sulfate (Bi X-Caps). As compared to conventional, nonradiopaque APA capsules, Ba X-Caps and Bi X-Caps containing human cadaveric islets resulted in a decrease in cellular viability of less than 5% up to 14 days after encapsulation. Both radiopaque capsules were found to be permeable to lectins $\leq$75 kDa, but were impermeable to lectins $\geq$120 kDa, thus ensuring the blockage of the penetration of antibodies while allowing free diffusion of insulin and nutrients. The glucose-responsive insulin secretion of the radiopaque encapsulated human islets was found to be unaltered compared to that of unlabeled controls, with human C-peptide levels ranging from 3.21 to 2.87 (Ba X-Caps) and 3.23 to 2.87 (Bi X-Caps) ng/islet at 7 and 14 days postencapsulation, respectively. Using fluoroscopy, both Ba X-Caps and Bi X-Caps could be readily visualized as single radiopaque entities in vitro. Furthermore, following transplantation in vivo in mice and rabbits, single capsules could be identified with no significant change in contrast for at least 2 weeks. This study represents the first attempt at making radiopaque microcapsules for X-ray guided delivery and imaging of cellular therapeutics. While human cadaveric islets were used as a proof-of-principle, these radiopaque capsules may have wide ranging therapeutic applications for a variety of cell types.

Keywords: Microencapsulation; alginate; X-ray; barium sulfate; bismuth sulfate; islet cells; diabetes

Introduction

Microencapsulation of therapeutic cells has provided a range of promising treatments for a number of diseases including type I diabetes,[1] hemophilia,[2] cancer,[3,4] Parkinson's disease,[5] and fulminant liver failure.[6] Microencapsulation creates a semipermeable membrane that prevents passage of antibodies and complement thereby preventing graft rejection.[7] While antibodies are blocked, the selective permeability of the capsule allows for passage of therapeutic factors

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produced by encapsulated cells. Some of the most convincing arguments for microencapsulation include the possibility of eliminating immunomodulatory protocols or immunosuppressive drugs while allowing for the long-term de novo delivery of therapeutic factors (drugs or cells) in either a local or systemic manner.

Recent advances in islet cell transplantation for type I diabetes mellitus (TIDM) have provided insulin-independence in patients through successful engraftment.[8] The restricted availability of cadaveric human donor pancreata, in conjunction with potential risks associated with immunosuppression, attenuate the impact of islet cell transplantation on clinical therapy of TIDM.[9] In addition to the health complications associated with chronic immunosuppressive therapy, many immunosuppressive regimens have been found to be selectively toxic to isolated islet cells.[10] For this reason, a method of transplanting allogeneic islets free of an immunosuppressive regimen would be ideal. Microencapsulation could potentially overcome the two major limitations in islet cell transplantation, namely, limited allogeneic pancreatic tissue availability and deleterious effects of chronic immunosuppresion. By enabling the immunoisolation of xenogenic grafts, microencapsulation would provide a means of transplanting a relatively inexhaustible source of islets such as porcine islets free of immunosuppresion.[11]

Before microencapsulation can be widely employed as a vehicle for transplantation of xenogenic or allogeneic cellular therapeutics, basic questions such as the ideal transplantation site, the best mode of delivery, verification of accurate delivery, and long-term survival of such grafts must be addressed.[12] If capsules could be detected by traditional imaging modalities, many of these questions could be better answered. To this end, we have developed two novel X-ray visible alginate microcapsules using barium sulfate (Ba X-Caps) and bismuth sulfate (Bi X-Caps). The radiopaque capsules are designed for imaging of cellular therapeutics with X-ray modalities (fluoroscopy, computed tomography (CT), and digital subtraction angiography). We present here the initial characterization of these novel microcapsules with assessment of permselectivity, insulin production and glucose responsiveness of encapsulated human islets, and demonstration of in vivo imaging following implantation in mice and rabbits.

Experimental Section

Cell Culture. Human islets isolated from a brain-dead donor were provided by the Joslin Diabetes Research Center (Boston, MA) under an approved protocol of the National Islet Cell Resource Program. Islets were cultured in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 1% penicillin/streptomycin/L-glutamine (all reagents from Sigma Co.) in a humidified CO_2 incubator at 37°C and 5% CO_2 atm. For microencapsulated cells, groups of 100 microcapsules, each initially containing ~1 islet, were cultured in tissue culture multiwell plates. Culture media was exchanged every 3 days.

Radiopaque Microencapsulation. The synthesis of radiopaque capsules was performed by modifying one step of the classic alginate/poly-L-lysine (PLL)/alginate (APA) microencapsulation protocol developed by Lim and Sun,[13] in which the PLL is a polycationic stabilizer for the microcapsules. In standard APA microcapsules, the positively charged amino group of the lysine molecule interacts with the negatively charged carboxyl groups of the uronic acid (basic unit of alginate). Protanal HF alginate from FMC Biopolymers (Haugesund, Norway) and Keltone HVCR alginate from Monsanto (St. Louis, MO) were first purified with filtration through a 0.2 μm pore size filter. Purified alginate was then utilized to microencapsulate human islets with an electrostatic droplet generator. Islet cells were first suspended in a solution of 2% w/v purified sodium Protanal HF alginate with either 5% w/v barium sulfate (Sigma, St. Louis, MO) or 5% w/v bismuth sulfate (Sigma, St. Louis, MO) added. Spherical droplets were formed by the electrostatic interaction coupled with syringe pump extrusion and were collected in a 100 mM calcium chloride solution. The gelled droplets were suspended in 0.05% poly-L-lysine (Sigma, molecular mass = 22−24 kDa). The droplets were washed with 0.9% saline and resuspended in 0.15% Keltone HVCR alginate for 5 min. Capsules were then washed with 0.9% saline. Unlabeled, nonradiopaque alginate poly-L-lysine (APA) capsules prepared without barium or bismuth sulfate were included as controls.

Cell Viability Assay. Following radiopaque encapsulation, the viability of human islets was determined by a microfluorometric assay. Encapsulated islet cells were incubated with 10 mM Newport Green (NG, Sigma, St. Louis, MO) for 30 min and 5 mM propidium iodide (PI, Sigma, St. Louis, MO) for 10 min. NG was excited using the 500 nm laser line, and the emitted fluorescence was detected through a 535 nm long-pass filter. PI was excited using the 514 nm

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laser line, and the emitted fluorescence was detected through a 550 nm long-pass filter. Red fluorescent (PI) cells were counted as dead, and green fluorescent (NG) were counted as viable. In cases of dual partial red and green staining, cells were counted as dead. Seven representative microcapsules from three independent preparations each (total of 21) were randomly selected for cell viability determinations. Random selection of microcapsules was accomplished by vortexing each tube just prior to pipetting a 100 μL sample from the tube onto a glass slide.

Permeability Assay and Microscopy. In order to determine the permeability of Ba X-Caps and Bi X-Caps as compared to APA controls, the microcapsule preparations were incubated with one of four fluorescently labeled lectins of varying molecular weight. Lectin incubation consisted of either incubation with 15 μL (1 mg/μL) of FITC-*Triticum vulgare* (WGA, molecular mass = 36 kDa), FITC-*Maackia amurensis* I (MAL-I, molecular mass = 75 kDa), FITC-*Ricinus communis* (RCA-I, molecular mass = 120 kDa), or FITC-*Sambuca nigra* (SNA, molecular mass =150 kDa). All lectins were obtained from EY Lab Inc. except FITC-*Maackia amurensis* I (Vector laboratories). Capsules were incubated for 48 h at 4 °C on a mechanical rocker, after which they were examined microscopically (Olympus ×51 and IX71 epifluorescence microscopes equipped with an Olympus DP-70 digital acquisition system) following embedding with Vectashield mounting medium (Vector, Burlingame, CA). Macroscopic images of microcapsules in 6 well plates were obtained with a D100 6MP Digital SLR Camera (Nikon; Melville, NY), and fluoroscopic images were obtained as described above.

Insulin Secretion Assay A static incubation assay was used to assess the insulin secretion response of encapsulated human islets. One hundred encapsulated islets were placed in a culture insert (membrane pore diameter 12 μm; Millicell PCF, Millipore, France) in 6 well plates. The insulin secretion was measured after 1.5 h in a solution of a specific glucose level. Specifically, a stepwise increase in glucose concentration from 6 mM to 8 mM D-glucose in RPMI 1640 medium was employed to assess the fine glucose responsiveness of encapsulated cells. Aliquots of the medium were stored at −80 °C. The C-peptide content of the samples was determined with an enzyme linked immunosorbent assay (ultrasensitive human c-peptide ELISA, Alpco Diagnostics, Windham, NH); results (in ng/mL) were expressed as the means of three independent experiments. The C-peptide secretion was also assessed at 7 days and 14 days following islet encapsulation using 8 mM glucose and 90 min incubation.

Transplantation in Mice and Rabbits. All animal studies were approved by our institutional animal care and use committee. Two female C57/BL mice (Charles River) age 6−8 weeks were used as recipients for microcapsules. Before transplantation, mice were anesthetized with ketamine (65 mg/kg ip, Pfizer) and xylazine hydrochloride (13 mg/kg ip, Bayer). The anesthetized mice were strapped in a supine position to a table, and a total of 5000 Ba X-Caps or Bi X-Caps was injected into the peritoneal cavity with a 20-gauge needle under fluoroscopic guidance. Fluoroscopic imaging was peformed immediately after injection.

A rabbit weighing approximately 4 kg was sedated with acepromazine (1 mg/kg) mixed with ketamine (40 mg/kg) intramuscularly (IM). An intravenous catheter was placed in the ear vein, and the rabbit was induced with thiopental (∼10 mg/kg to effect). The rabbit was then intubated to maintain an open airway. General anesthesia was maintained with intravenous thiopental. The anesthetized rabbit was placed in a supine position on a table, and a total of 2000 Ba X-Caps and 2000 Bi X-Caps were injected IM under fluoroscopic guidance in the hind limb of the rabbit. Fluoroscopic imaging was performed immediately and at 2 weeks postinjection.

X-ray Imaging. Fluoroscopic imaging was performed using a Toshiba Infinix VC-i unit with imaging settings of 64 kVP (kVP = kilovolt peak), 66 ms exposure time, 112 mA tube current, and 910 mm SID. The image intensifier size was 5 in. for the well plate and mouse images and 9 in. for the rabbit images.

Statistical Analysis. Statistical analysis was conducted using a Student's T test with a significance level $P < 0.05$. Data were also analyzed using the bioequivalence (BE) test. The test was performed using the two one-sided T-test approach (TOST).[14] In a BE test, the null hypothesis is that two groups *differ* by an amount θ or more. In TOST, the null hypothesis is rejected and two groups are declared bioequivalent at the type I error rate α if a $(1 - 2\alpha)$ confidence interval is contained in $(-\theta, \theta)$. Because no θ value has been established for declaring bioequivalence in encapsulated cell viability, we report the lowest value that would allow the two samples to be declared bioequivalent, with θ being reported as a percent difference from control. All statistical analysis was done using the statistical software R.

Results

Islet Encapsulation and Capsule Permeability. After proper adjustment of the concentration of islets in alginate, we were able to encapsulate approximately one islet equivalent per capsule as visualized by DAPI labeling and fluorescence microscopy (Figure 1B). Immediately following the encapsulation process, islet recovery was 90 ± 4% (n = 14 independent encapsulation procedures). The total volume of encapsulated human islets was calculated in a 15 mL Falcon centrifuge tube. The mean volume of 1000 encapsulated and nonencapsulated islet equivalents (IE) was 40 ± 3 μL and 3.6 ± 0.4 μL, respectively. Capsules had a characteristic white (Ba X-Caps) or yellow (Bi X-Caps) macroscopic appearance (Figures 1A and 4A,C), with an average diameter of approximately 350 μm (Figure 1B,C). As compared to the unlabeled microcapsules, the radiopaque

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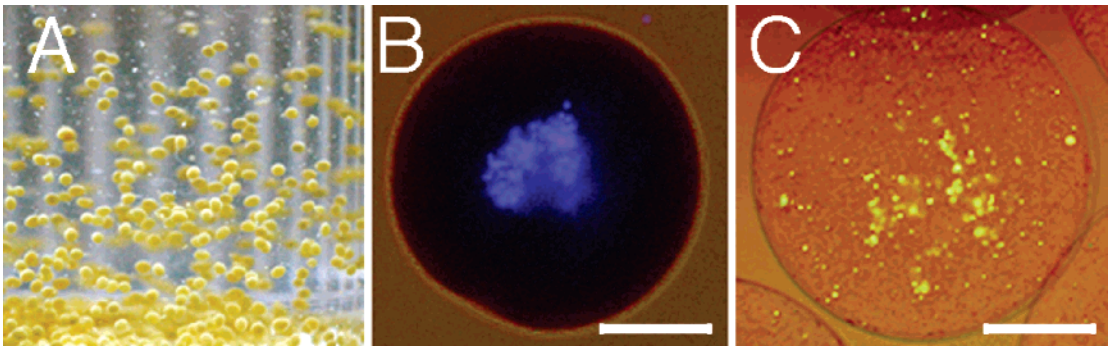

Figure 1. **(**A) Macroscopic image of Bi X-Caps. (B) Microscopic image of a Bi X-Cap with an encapsulated human islet stained with DAPI nuclear stain. (C) Microscopic image of a APA microcapsule after incubation with 75 kDa fluorescent lectins demonstrates intracapsular fluorescence, demonstrating capsule permeability for molecules ≤75 kDa. Bar = 100 μm.

Table 1. Permeability of Ba X-Caps, Bi X-Caps, and Unlabeled, Nonradiopaque APA Controls for Fluorescent Lectins with Various Molecular Masses[a]

molecular mass (kDa)	barium	bismuth	control
36	√	√	√
75	√	√	√
120	×	×	×
150	×	×	×

[a] √ = permeable. × = nonpermeable.

capsules exhibited a much higher weight density and settled rapidly in solution.

Unlabeled (nonradiopaque) APA microcapsules, Ba X-Caps, and Bi X-Caps were found to have equal permeability

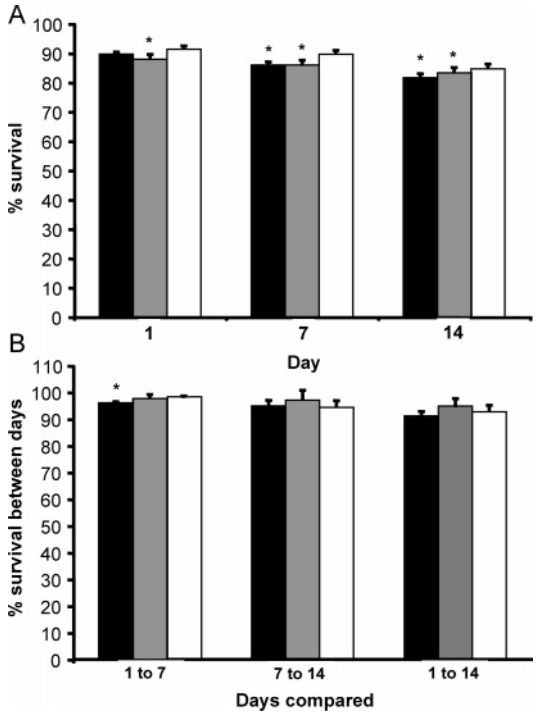

Figure 2. (A) Percentage survival of human islets encapsulated in Ba X-Caps (black bars), Bi X-Caps (gray bars), and unlabeled, nonradiopaque capsules (white bars). (B) Percentage survival between 1 and 7, 7 and 14, and 1 and 14 days. * = statistically significant different from control capsules ($p < 0.05$).

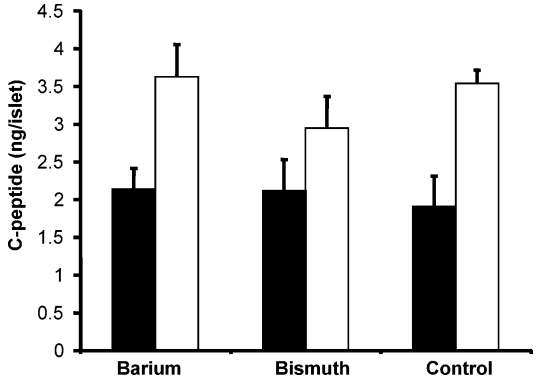

Figure 3. C-peptide secretion of human islets encapsulated in Ba X-Caps, Bi X-Caps, and unlabeled, nonradiopaque control capsules after 90 min incubation in a 6 mM (black bars) and an 8 mM (white bars) glucose solution. C-peptide secretion was not significantly different between radiopaque capsules and control capsules.

to fluorescent lectins (Table 1). An illustration of the permeability of a Bi X-Cap for lectins with a molecular mass of 75 kDa is shown in Figure 1C. Specifically, all capsule types were found to be permeable to fluorescent lectins ≤75 kDa but were found to be impermeable to lectins ≥120 kDa, thus blocking antibodies while allowing penetration of smaller nutrients and secretion of insulin (molecular mass ~ 5 kDa).

Islet Viability. The difference in viability of human islets encapsulated in Ba X-Caps and Bi X-Caps as compared to unlabeled APA microcapsules was assessed at days 1, 7, and 14. The percentage survival for each day is shown in Figure 2A. The percentage survival of day 1 compared to day 7, day 7 compared to day 14, and day 1 compared to day 14 is shown in Figure 2B. In general, the survival from day 1 for the Ba X-Caps and Bi X-Caps was shown to have a minor although statistically significant difference ($p < 0.05$). The result of the BE test showed that these differences were indeed minor ($\theta < 10\%$ for all cases). Interestingly, with the exception of Ba X-Caps between days 1 and 7, the change in viability that occurred between days showed no statistically significant difference ($p > 0.05$) (Figure 2B), with a BE test θ value <10%.

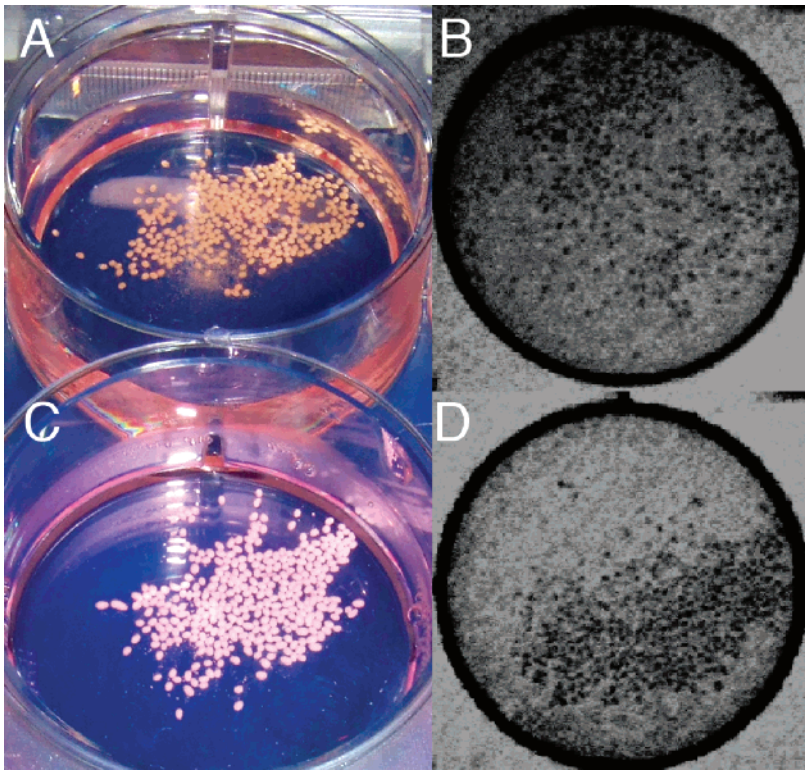

Figure 4. Macroscopic (A, C) and fluoroscopic (B, D) images of (A, B) Bi X-Caps and (C, D) Ba X-Caps. Single capsules can be clearly identified. Note the difference of color appearance between the two capsule preparations.

Insulin Production and Glucose Responsiveness. The insulin secretory response of islets in Ba X-Caps and Bi X-Caps was compared against unlabeled, nonradiopaque APA microcapsules. In order to detect any specific difference in insulin secretion from human islets in the two capsule types, encapsulated islets were incubated in solutions of 6 and 8 mM glucose. One day after encapsulation, no statistically significant difference ($p < 0$ 0.05) in insulin secretion was found between the radiopaque and nonradiopaque encapsulated islets after incubation in 6 and 8 mM glucose solutions (Figure 3). The glucose responsiveness stimulation index as defined by the increase in insulin secretion after changing from 6 to 8 mM glucose solution was 1.69, 1.59, and 1.90 for Ba X-Caps, Bi X-Caps, and unlabeled capsules, respectively (Figure 3). The magnitude change in insulin response was analyzed (1 − (6 mM [c-peptide]/8 mM [c-peptide])). There was no statistically significant difference in the magnitude change ($p > 0.05$) from control with both barium and bismuth.

To assess changes in insulin production over time, c-peptide secretion from human encapsulated islets was also assessed for each capsule preparation over 90 min in an 8 mM glucose solution after 7 and 14 days in culture. The c-peptide secretion (ng/islet) from encapsulated islets at 7 and 14 days was found to be respectively 3.21 and 2.87 ng/islet for Ba X-Caps, 3.23 and 2.95 ng/islet for Bi X-Caps, and 3.53 and 3.03 ng/islet for unlabeled capsules.

Imaging of Microcapsules. The radiopaque labeling of the APA capsules enabled fluoroscopic imaging and noninvasive X-ray tracking of encapsulated islets in mice and rabbits. Individual, single Ba X-Caps and Bi X-Caps could be easily visualized in vitro in normal saline in a multiwell plate (Figure 4). Using standard fluoroscopy, real time X-ray-guided delivery of both capsule types in mice and rabbits was possible. Individual, single Ba X-Caps and Bi X-Caps were clearly identified in vivo after transplantation into the peritoneal cavity of mice (Figure 5), and after intramuscular injection into the hind limb of rabbits (Figure 6). Two weeks after injection, both X-Caps retained their radiopacity in vivo as compared to day 0 (compare Figure 6 top row with bottom row).

Discussion

We have developed, for the first time, radiopaque capsules that can be used for encapsulation of cells and allow real time X-ray guided delivery as well as noninvasive X-ray followup imaging. Our capsules offer immunoprotection (Table 1) and thus may prevent initial graft rejection of cellular therapeutics following transplantation. The synthesis of the radiopaque microcapsules is based on a modification of the classic alginate/poly-L-lysine/alginate (APA) microencapsulation protocol developed by Lim and Sun.[13] In order to make microcapsules of diameters <350 μm (Figure 1A), an electrostatic droplet generator[15] was substituted for a traditional air-droplet generator to encapsulate human islet cells. We modified the traditional synthesis of capsules by

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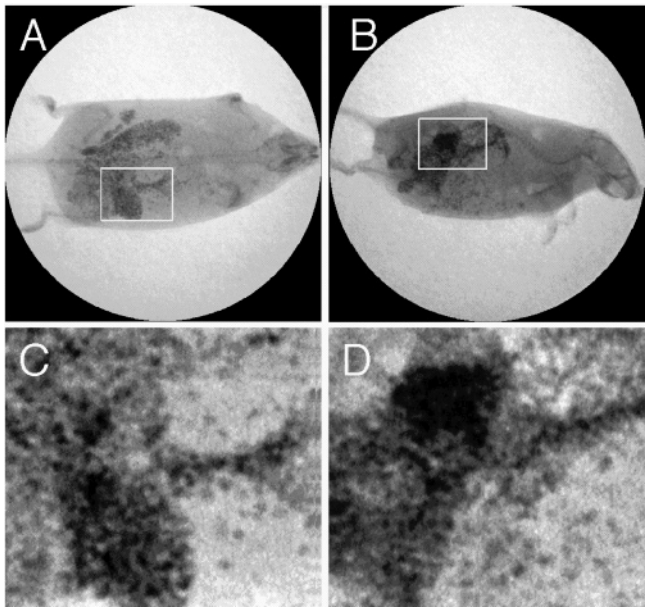

Figure 5. In vivo imaging of X-Caps following transplantation in the peritoneal cavity of mice. (A, C) Fluoroscopic image following injection of 5000 Ba X-Caps. (B, D) Fluoroscopic image following injection of 5000 Bi X-Caps. (C, D) Enlargement of boxed areas in panels A and B.

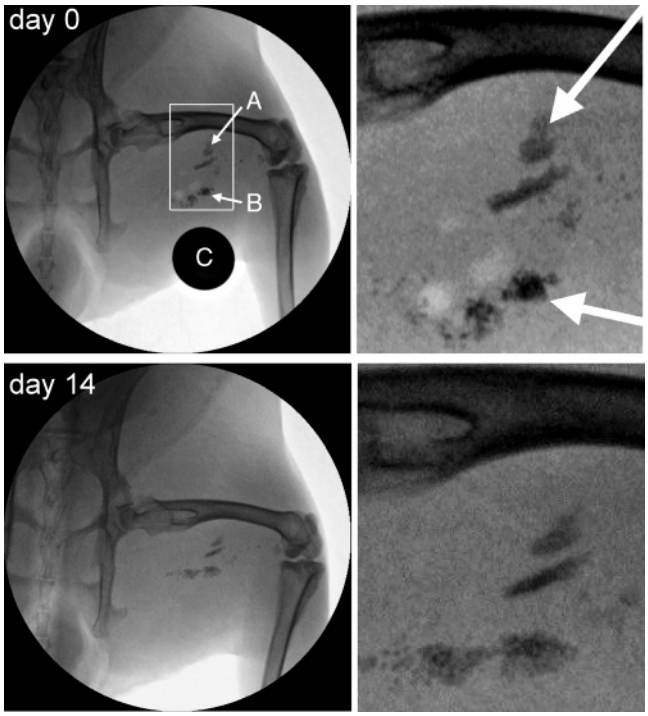

Figure 6. In vivo imaging of X-Caps immediately (top row) and 2 weeks (bottom row) after intramuscular transplantation into a rabbit hindlimb. A = 2000 Ba X-Caps; B = 2000 Bi X-Caps; C = quarter for reference of size and opacity. Magnification of fluoroscopic image is shown on right.

adding barium or bismuth to the inner core layer of high guluronate alginate (Protanal) that surrounds the islet. The outer layer of the APA capsule, made with high mannuronate alginate (Keltone), was added without contrast in order to avoid any potential inflammatory or toxic reaction induced by the radiopaque contrast agents. We have chosen a high guluronate alginate for the inner alginate layer because of its superior strength.[16] For the outer layer we chose a high mannuronate alginate as it has been shown to be less immunogenic.[17,18] Although barium and bismuth are heavy metals, and their water soluble compounds can potentially be toxic, the extremely low solubility of barium and bismuth sulfate protects the encapsulated cell graft and the transplant recipient from rapidly absorbing harmful amounts of the metals.

For an immunoisolation device to function properly, its permeability is of critical importance. It was conceivable that the incorporation of the contrast agents may have altered the permeability of the microcapsules. The contrast agents might occlude some of the channel space available for solute diffusion, hence increasing the permeability threshold. However, this proved not to be the case (Table 1).

Previous methods for tracking of cellular therapeutics without reporter genes have been limited to direct labeling of cells with radioisotopes (i.e., ^{111}In−oxine)[19,20] or magnetic resonance (MR) contrast agents[20−22] for single photon emission computed tomography (SPECT) and MR imaging, respectively. Here we present the first method of tracking cellular therapeutics using X-ray modalities (i.e., fluoroscopy, CT, and digital subtraction angiography). By encapsulating cells in radiopaque alginate microcapsules, we potentially avoid many of the limitations involved with direct labeling of cells. As opposed to the majority of methods of cellular magnetic labeling for tracking with MR imaging which involve prolonged incubation of cells with contrast agents,[23] X-ray caps can be synthesized in under an hour. In addition, an inherent limitation of intracellular labeling is the resulting dilution effect when cells divide. In our approach, as capsules and not the cells contain contrast agent, this dilution effect should not occur even in the presence of rapid cell division within the microcapsule. Furthermore, adding contrast agent

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to the capsule instead of intracellular labeling of cells may bypass potential toxicity issues that result from direct cell labeling. For instance, a high payload of cellular ^{111}In−oxine can lead to radiotoxicity and cell death,[19] while iron oxide labeling of cells may interfere with certain stem cell differentiation pathways.[24] With regard to direct labeling of pancreatic islet cells with superparamagnetic iron oxide MR contrast agents, in vivo glucose stimulation experiments resulted in a significant (i.e., 50%) reduction in insulin secretion, compared to unlabeled cells.[25] Finally, in addition to providing a means of tracking, radiopaque capsules provide immunoisolation, thus potentially preventing rejection of cellular therapeutics in an immunohostile environment.

The in vivo imaging results in mice and rabbits (Figures 5 and 6) demonstrated that the capsules can be readily visualized at a single capsule level. Thus, our Ba X-Caps and Bi X-Caps represent the first method of delivering cellular therapeutics with significant radiopacity to assess cellular position. Previous studies have relied on a test injection of radiopaque contrast agents prior to cellular therapies to verify the injection location, but the ability to track the cell location serially on the basis of the radiopaque agent was not possible.[26] Radiopaque contrast agents encapsulated in alginate (X-Caps) could overcome this limitation. Both in mice and in rabbits, we found the capsular contrast to be persistent for at least 2 weeks. Both barium and bismuth have extremely low water solubilities, which largely explains their long-lasting contrasting properties. Further studies are needed to assess the long-term durability of radiopacity and also the potential of X-ray imaging modalities to detect changes in contrast and cell survival when capsules rupture.

In this initial study using X-Caps, we injected our preparations in the peritoneal cavity as it has been extensively used as transplantation site for encapsulated islets. The peritoneal cavity contains sufficient space to accommodate the total capsular volume and facilitates insulin access to the liver (which is the major organ for insulin consumption after release). Previous studies have shown that total microencapsulated islet transplant volumes of an average of 80 mL per dog and 120 mL per human patient[27] are necessary to achieve a sufficient, functional islet cell mass. However, the peritoneal cavity may ultimately turn out not to be the best transplantation site. For instance, the reduced oxygen supply and the highly reactive nature of the peritoneal cavity to foreign bodies and antigens are a drawback.

Other groups have explored making nonalginate hydrogel microcapsules radiopaque for use as embolic agents. Contrast agents explored to date include tantalum powder or tungsten powder,[28] iothalamic acid, and iopanoic acid.[29,30] In addition to these agents we explored the addition of numerous other agents to the primary layer of alginate in APA microcapsules. Radiopaque contrast agents tested for inclusion in the primary layer of alginate microcapsules included MD-Gastroview (diatrizoate sodium and diatrizoate meglumine), Omnipaque (iohexol), Hypaque (diatrizoate sodium and diatrizoate meglumine), manganese chloride, and potassium iodide. In all cases, contrast was not stably incorporated within the microcapsule but instead was eluted over a period of hours. We did find that tantalum and tungsten powder could be readily encapsulated and retained within alginate microcapsules but the level of contrast from these agents was greatly reduced compared to barium and bismuth. Though not currently approved for parenteral use, preparations of barium or bismuth complexed with stabilizing molecules may prove to be highly efficacious and have good relative safety compared to currently employed agents. A recent report on the safety and efficacy of a novel bismuth sulfide nanoparticle CT contrast agent compared to traditional iodinated contrast agents supports this claim.[31]

An important consideration with our radiopaque contrast capsules is whether the radiopaque labeling interferes with the functionality of the encapsulated cells. This question was addressed in two ways. First, the cell viability was analyzed, and second, the insulin secretion and glucose responsiveness were determined. The cell viability data (Figure 2A) suggest that there are very minor differences in cell viability between the capsule preparations. The data in Figure 2B show that these differences further decrease with time. The results for the glucose response and insulin secretion of the two X-Caps showed no statistically significant difference between the radiopaque and control capsules.

As the radiopaque contrast agents are added to the primary alginate layer, our labeling strategy could also be used for other alginate microcapsule preparations including those that substitute chitosan[32] and poly-L-ornithine[33] in place of poly-L-lysine, or add additional compounds such as poly(ethylene glycol).[34] Further characterization is clearly warranted to assess potential applications for a variety of cellular therapeutics, drug delivery, and embolization applications. Be-

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cause the biophysiological environment between cultured encapsulated cells in vitro and transplanted ones in vivo can be quite different, future studies evaluating the viability and function of encapsulated cells in animals is warranted. While we have chosen to use encapsulated human islets as a proof-of-principle because they have been used in preclinical[35] and clinical[36,37] studies with remarkable success to manage or cure diabetes, Ba X-Caps and Bi X-Caps can in principle be applied to track, by X-ray, a wide variety of cellular therapeutics. These could include, but are not limited to, encapsulated stem cells producing regeneration-promoting soluble factors, encapsulated GDNF-producing cells to treat Parkinson's disease,[38] or encapsulated endostatin-producing cells to inhibit tumor angiogenesis.[39,40]

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