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Sterilization of Silastic Capsules Containing 17β-Estradiol for Effective Hormone Delivery in *Mus musculus*

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Silastic capsules are frequently used to study the physiologic effects of estrogen exposure in animal models. The Office of Laboratory Animal Welfare requires the sterilization of nonpharmaceutical-grade compounds before use. We compared 2 commonly used terminal sterilization methods—ionizing radiation (IR) and ethylene oxide (EO)—for their utility in sterilizing silastic capsules containing 0.05 or 0.1 mg 17β-estradiol (E2). E2-specific ELISA demonstrated that serum estrogen levels did not differ between mice implanted with 0.05-mg E2 capsules that were sterilized with IR or EO and those implanted with nonsterilized capsules. Likewise, mammary gland morphology and progesterone receptor expression and proliferation in mammary epithelium were similar among mice treated with E2 capsules, regardless of sterilization method, and pregnant day 15 mice. In addition, IR-sterilized 0.1-mg E2 pellets provided high serum E2. We conclude that neither ionizing radiation nor ethylene oxide degraded E2 or the cellulose matrix, suggesting that these methods of sterilization are appropriate to provide effective sterile hormone capsules for animal research.

Abbreviations: BrdU, bromodeoxyuridine; E2,17β-estradiol; EO, ethylene oxide; IR, ionizing radiation; OLAW, Office of Laboratory Animal Welfare; Pgr, progesterone receptor

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Research to demonstrate the physiologic effects of estrogen exposures frequently relies on the use of animal models. Methods for estrogen delivery in animals include routine injections, oral gavage, commercially prepared pellets, and the implantation of silastic capsules. Silastic capsules offer flexibility in dosage and are convenient for sustained delivery of estrogen. Database searches for the preparation of silastic capsules identified more than 860 citations and indicate that this hormone delivery method is commonly used in a variety of research applications for hormonal treatment of mice, rats, reptiles, fish, birds, and NHP.[1,3,10,13,19] In 2011, the Office of Laboratory Animal Welfare (OLAW) implemented requirements for use of pharmaceuticals in animals. The 2011 OLAW Guide[9] states, "Pharmaceutical-grade chemical compounds should be used, when available, for all animal-related procedures." The use of nonpharmaceutical grade substances in animals must be approved by the IACUC and often requires demonstration that these compounds are reasonably safe in terms of grade, purity, pH, and sterility.[9] However, although PubMed identifies more than 30 references using silastic capsules since the publication of the 2011 OLAW Guide, none describe any methods used to ensure sterility.

Commercially prepared hormone pellets offer several advantages over other methods of hormone delivery in rodent animals. The first advantage is that these pellets are provided sterile. Other advantages are that these pellets are marketed to deliver consistent uniform subcutaneous release of hormone for a specified length of time. However, our group and others have found these commercially prepared pellets elevate serum hormone levels to a greater extent than projected, and hormone delivery is not consistent.[8,17] In addition, purchasing pellets limits the flexibility of experimental design, involves placing required minimum orders for each dose, and are cost-prohibitive.

Preparing hormone compounds in reagent-grade oils for subcutaneous injection offers flexibility for testing doses, and sterility can be achieved through filtering or dry-heat sterilization. However, for experiments that require long-term administration of hormones, repeated handling of rodents can be disadvantageous, especially because rodents that receive daily injections with hormones suspended in oils can develop skin lesions.[14] Although oral treatment with compounds mixed in oil or nut butter do not require sterilization, repeated handling of mice for gavage comes with some risk for esophageal damage or accidental injection in lungs. Dietary treatment requires intake monitoring. In addition, orally administered natural and synthetic estrogens may have low bioactivity and systemic activity or elevated hepatic estrogenic toxicity.[5]

We have found that hormones packed in silastic capsules are convenient for extended delivery in mice because insertion or removal under isoflurane anesthesia is easy and quick. Because the silastic hormone capsules are prepared in the lab, hormone concentrations are easily tested and adjusted. However, concern exists that sterilization methods may degrade hormones. Although 17β-estradiol (E2) is stable when stored in ambient conditions devoid of light, E2 shows exponential photodegradation over time,[2] suggesting that it may also be vulnerable to sterilization methods. In the current study, we demonstrated that silastic estrogen capsules can be sterilized by using ionizing radiation (IR) or ethylene oxide (EO) without

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diminishing efficacy; therefore these methods can be used to satisfy OLAW and IACUC sterility requirements for estrogen-containing silastic implants. More importantly, these methods for sterilization of hormone capsules are consistent with the practice of aseptic surgical technique to prevent infection and ensure consistent scientific results.

Materials and Methods

Animal treatments. Female BALB/c mice (weight, 20 to 25 g) were housed in a temperature- (70 °F) and humidity- (40%) controlled rodent vivarium under a 12:12-h light:dark cycle. Mice (3 per cage) were housed in cages with isolator tops and ventilated racks that were changed every 14 d. All procedures were in accordance with protocols approved by the University of Massachusetts IACUC. All mice received a commercial chow (no. 5058, LabDiet, St Louis, MO) and water without restriction. At 6 wk of age, mice were anesthetized by using 2.5% isoflurane and were implanted subcutaneously with either a silastic capsule or a 14-d release commercial pellet (Innovative Research of America, Sarasota, FL) between the scapulae; in some cases, capsules or pellets were removed 14 d after implantation. At 2 h before euthanasia, mice were intraperitoneally injected with BrdU (70 µg/g body weight; catalog no. B5002, Sigma Aldrich, St Louis, MO) prepared in sterile PBS at 10 mg/mL. After euthanasia, tissues were collected, including whole blood through cardiac puncture. Blood was allowed to coagulate at room temperature and then was centrifuged at 4600 × *g* for 5 min to separate serum. Fourth or inguinal mammary glands were collected for RNA, whole mounting, or immunohistochemistry. The lymph node was removed from the mammary glands intended for RNA isolation, and then flash-frozen on dry ice. Mammary glands were prepared for whole mounts or fixed in 10% neutral buffer formalin for histology.

For use as positive controls, female mice (age, 5 wk) were mated to produce parous dams. After parturition, dams nursed for 4 d, after which the litters were removed and the dams allowed to involute for 28 d. At the time of tissue harvest, parous mice were 14 to 15 wk of age. In addition, tissue samples were collected from age-matched virgin mice.

Preparation and sterilization of silastic capsules. Capsules were made by using silastic tubing (inner diameter, 1.90 mm; outer diameter, 3.18 mm; length, 1.2 cm; catalog no. 508-009, Dow Corning, Midland, MI) sealed with silicone (catalog no. 070798006881, DAP, Dow Corning). They were packed with cellulose (catalog no. AC382312500, Fisher Scientific, Waltham, MA) or E2 (0.05 or 0.1 mg; catalog no. E27858, Sigma Aldrich) solubilized in ethanol to produce the desired concentration and then mixed with cellulose to fill the capsule. Capsules were sterilized by using EO or 5 kGy ^{137}Cs irradiation or were left unsterilized. Sterilized capsules were preprimed for 24 h in DMEM:F12 phenol red–free media (Sigma Life Sciences, St Louis, MO) at 37 °C prior to implantation. Priming media can be used in E2 ELISA to gauge hormone secretion or to assess for microbial contamination.[16]

Hematoxylin and eosin staining and BrdU assay. Paraffin-embedded 4-µm sections were deparaffinized and stained with hematoxylin and eosin on an autostainer (Dako, Carpinteria, CA). BrdU incorporation analysis was performed by using BrdU Staining Kit (catalog no. 93-3943, Invitrogen, Carlsbad, CA). Images were taken at a magnification of 200× (model BX40 microscope, Olympus, Tokyo, Japan) by using a MicroPublisher 3.3RTV camera (QImaging, Surrey, British Columbia, Canada). Proliferating cells were quantified (ImageJ software, https://imagej.nih.gov/ij/) as the percentage of BrdU-positive nuclei among 600 total cells.[18]

Preparation of whole-gland mounts. Mammary glands were pressed between 2 glass slides and fixed in Carnoy fixative (ethanol:chloroform:glacial acetic acid, 6:3:1). Rehydrated mammary glands were stained overnight in carmine alum solution (2 g/L carmine alum in 10 mM aluminum potassium sulfate). Tissues are dehydrated in a graded ethanol series and cleared in xylenes before mounting on slides (Permount, Fisher Scientific). Images were obtained under 40× magnification (model BZ-X700 microscope, Keyence, Itasca, IL).

qPCR analysis. RNA from flash-frozen 4th mammary glands was isolated by using TRIzol (catalog no. 15596018, ThermoFisher Scientific, Waltham, MA), cDNA was prepared by using Protoscript II First Strand cDNA Synthesis Kit (catalog no. E6560S, New England BioLabs, Ipswich, MA), and qPCR analysis of the progesterone receptor (*Pgr*) gene (right primer, GGTGGGCCTTCCTAACGAG; left primer, GACCACATCAGGCTCAATGCT) was performed in a thermocycler (model no. CFX96 Real-Time System, Bio-Rad, Hercules, CA).

Radioimmunoassay. Serum E2 levels were calculated by using an E2-specific radioimmunoassay ImmuChem Double Antibody 17β-estradiol ^{125}I RIA Kit, ICN Biomedicals, CA).

ELISA. Serum E2 levels were quantified by using an E2-specific ELISA kit (Calbiotech, San Diego, CA).

Statistical analysis. Statistical analyses were performed by using Excel (Microsoft, Redmond, WA), and significant differences were determined by using the 2-tailed Student *t* test. Significance was defined as a *P* value of 0.05 or less.

Results

Hormone release from commercial pellets. Commercially available hormone pellets do not require sterilization and are marketed to deliver consistent uniform subcutaneous release of hormone for a specified length of time. We have used 14-d pellets containing E2 (0.05 mg) with progesterone (0.3 mg) to mimic the effects of pregnancy on the mouse mammary gland.[4] Serum E2 levels in normally cycling mice range between 5 and 60 pg/mL and do not differ between age-matched virgin (46.5 ± 5.6 pg/mL) and parous mice (31.4 ± 8.8 pg/mL; Figure 1). To mimic the serum E2 levels of pregnant mice, we aim to maintain E2 levels between 60 and 110 pg/mL continuously from day 1 to day 14 after implantation (Figure 1; Day 15 pregnant, 65.9 ± 9.5 pg/mL). The commercial implants led to a considerable increase in serum E2 between days 1 and 14; the level on day 3 (1484 ± 507 pg/mL) was more than 20-fold greater ($P < 0.0001$) than target concentrations. We expected that by day 28, the estrogen in the 14-d commercial pellet would be completely depleted and that serum E2 would return to hormone levels associated with normal estrous cycling. However, the 0.05-mg pellets continued to deliver high concentrations of E2 for longer than 14 d, and E2 levels exceeded physiologic levels at both 28 d (339 ± 87, $P < 0.0001$) and 56 d (208 ± 54 pg/mL, $P < 0.01$) after implantation. Removing the pellet at day 14 was required to allow normal estrous cycling to resume as measured on day 28 after implantation (41.8 ± 11.3 pg/mL). After removal of the pellet, serum E2 levels did not differ among parous, age-matched virgin, and placebo-treated mice.

Effects of sterilization of E2-containing capsules on hormone efficacy. Prior to the OLAW requirement for sterilization, we used silastic hormone capsules to deliver E2 to both intact and ovariectomized mice. To ensure that the E2 capsules functioned and provided sufficient hormone after sterilization ,we used an E2-specific ELISA kit to compare serum E2 from mice implanted

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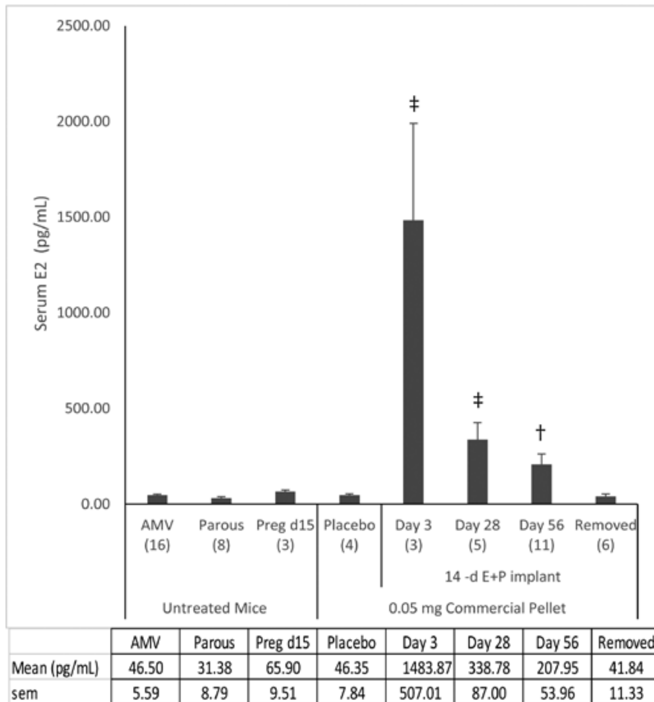

	AMV	Parous	Preg d15	Placebo	Day 3	Day 28	Day 56	Removed
Mean (pg/mL)	46.50	31.38	65.90	46.35	1483.87	338.78	207.95	41.84
sem	5.59	8.79	9.51	7.84	507.01	87.00	53.96	11.33

Figure 1. Effect of implanted commercial pellets containing 0.05 mg 17β-estradiol (E) and 0.3 mg progesterone (P) on serum estradiol (E2; mean ± 1 SEM [error bars]) levels in mice. Serum was collected from mice at 3, 28, and 56 d after implantation and at 28 d after implantation in a group of mice from which the pellets were removed after 14 d. Controls included serum collected from mice at 28 d after implantation of a commercial placebo pellet, from untreated control age-matched virgin (AMV) mice, control parous, and control pregnant day 15 mice. Serum E2 levels were determined through E2-specific radioimmunoasay. The number of animals per group is indicated in parenthesis; values significantly (*, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$) different from controls are indicated.

with silastic capsules that were sterilized through exposure to either 5 kGy of IR or to EO or left unsterilized (Figure 2). Control capsules were filled with a cellulose matrix and exposed to either 5 kGy IR or EO. E2 capsules sterilized by 5 kGy IR generated day-14 E2 serum levels of 70.5 ± 19.7 pg/mL; EO-sterilized capsules yielded 68.5 ± 22.2 pg/mL on day 14. These serum E2 levels were equivalent to those from the nonsterilized E2 capsules on day 14 (71.0 ± 21.3 pg/mL) and were greater ($P < 0.05$) than those of control mice implanted with empty or cellulose-filled control capsules (collective mean, 9.1 ± 2.4 pg/mL).

We used whole mounts of the mammary gland to visually evaluate silastic capsule–induced epithelial ductal branching and developmental differences in alveoli. Minimal morphologic differences were observed in the mammary glands of nulliparous control animals and those implanted with IR- or EO-sterilized control capsules for 14 d (Figure 3 A through C). Treatment with unsterilized E2 silastic capsules for 14 d promoted ductal outgrowth and alveolar development mimicking that of pregnancy at day 15 (Figure 3 E and D). Both IR- and EO-sterilized E2 capsules produced mammary glands with alveolar development that resembled the mammary glands from pregnant mice and those from mice implanted with the unsterilized E2 capsules (Figure 3 F and G).

We then quantified the relative expression of the progesterone receptor gene in mammary glands. *Pgr* expression is induced by estrogen and upregulated during pregnancy in the mammary epithelium.[6] Regardless of the sterilization technique, E2 capsules supported an increase (approximately 2-to 3-fold) in

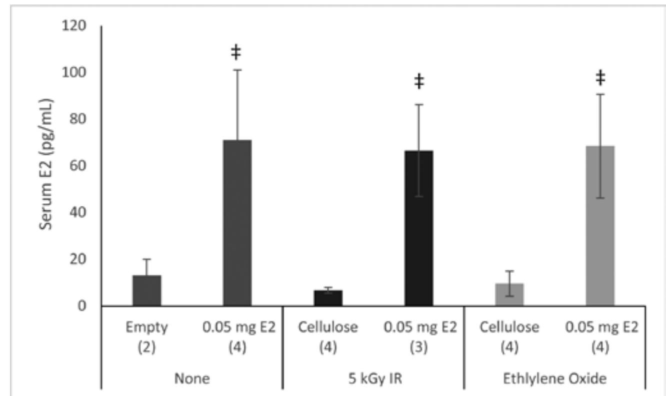

Figure 2. Quantification of serum E2 (mean ± 1 SEM [error bars]) induced by 14-d treatment with sterilized silastic hormone capsules. Mice were implanted subcutaneously with empty silastic capsules or silastic capsules containing cellulose (control) or 0.05mg E2. Capsules were sterilized by using ethylene oxide or 5 kGy irradiation (IR) or were left unsterilized. The number of animals in each group is indicated in parentheses. Serum was collected on day 14 of hormone treatment. Serum E2 levels were calculated by using E2-specific ELISA. Values significantly (‡, $P < 0.001$) different from control values are indicated.

relative *Pgr* gene expression on day 14 that corresponded to E2 serum levels (Figure 4). Both 0.05-mg E2 IR-sterilized capsules and 0.05-mg E2 EO-sterilized capsules enhanced *Pgr* expression in the mammary gland (2.7 ± 0.7 and 3.3 ± 1.3, respectively, $P < 0.05$) relative to combined controls. The level of *Pgr* expression did not differ between sterilization methods.

Serum E2 levels over the 14-d treatment period and associated responses in mammary gland. After the 14-d treatment period, serum E2 levels were elevated overall, and mammary glands generally exhibited typical estrogen-induced branching morphology, regardless of preimplantation sterilization (or not) of the silastic capsule. However, in some mice, 0.05-mg E2 capsules only partially induced ductal branching and alveolar development. In addition, serum E2 levels from the mice implanted with 0.05-mg E2 silastic capsules ranged from 15.1 to 125.9 pg/mL, such that several mice fell below an E2 threshold of 100 pg/mL on day 14. To assess whether 0.05 mg E2 was sufficient to sustain pregnancy levels of serum for the 14-d period, we compared IR-sterilized 0.05- and 0.1-mg E2 capsules (Figure 5 A) on days 3, 6, 9, and 12 through 14. On days 3 and 6 after implantation, 0.05-mg E2 capsules resulted in E2 serum concentrations (322 ± 15 and 249 ± 20 pg/mL, respectively) that exceeded (3.5- to 5-fold) those associated with pregnancy However, by day 9, serum E2 levels had dropped such that they were at or slightly below levels seen during pregnancy and had reached levels consistent with normal estrous cycling. These results suggest that 0.05-mg IR-sterilized E2 capsules may not provide sufficient hormone to sustain pregnancy concentrations for 14 d or to generate the appropriate physiologic responses. In comparison, 0.1-mg E2 capsules provided significantly higher serum levels of E2 throughout the 14-d treatment (0.1 mg relative to 0.05 mg at day 12 to 14, $P < 0.05$), albeit concentrations were as much as 20-fold greater on day 3 (1223.96 ± 83.20 pg/mL) and still 5-fold greater by day 12 to 14 (303.18 ± 44.93 pg/mL). In addition, we assessed relative *Pgr* expression levels at various time points as a surrogate for estrogen response in mammary gland (Figure 5 B). *Pgr* gene expression in mammary glands from mice treated with 0.05-mg E2 capsules was significantly ($P < 0.05$) increased relative to controls at all time points. This increase in gene expression peaked at day 3 (7.5-fold) and decreased to 2.6-fold on

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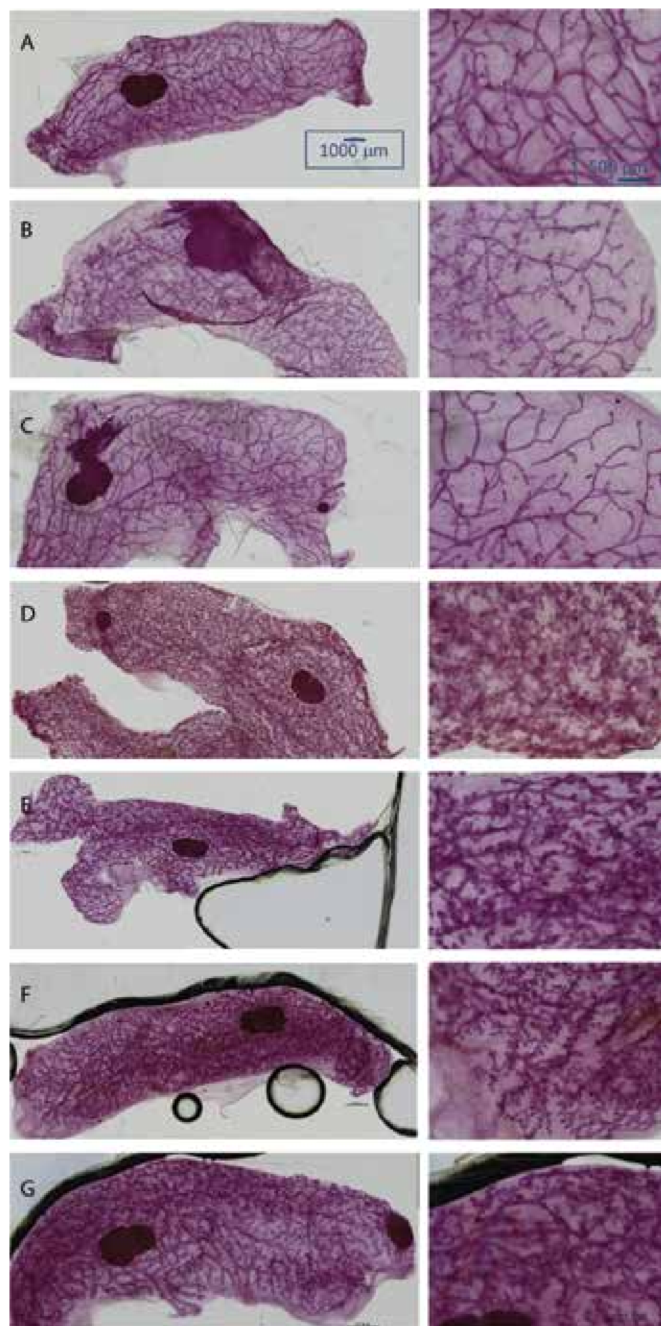

Figure 3. Morphologic changes in mammary glands induced by 14-d treatment with sterilized silastic capsules containing E2. Samples of the 4th abdominal mammary glands were fixed with 10% neutral-buffered formalin and stained with carmine alum. (A) Hormone-free nulliparous control mouse. (B) Implanted with IR-sterilized cellulose control capsule. (C) Implanted with EO-sterilized cellulose control capsule. (D) Day 15 pregnant control. (E) Implanted with unsterilized capsule containing 0.05 mg E2. (F) Implanted with IR-sterilized 0.05-mg E2 capsule. (G) Implanted with EO-sterilized 0.05-mg E2 capsule. Scale bar: 1000 µm (whole-gland images); 500 µm (magnification, 40×) otherwise.

days 12 through 14. In mice implanted with 0.1-mg E2 capsules, *Pgr* expression was significantly ($P < 0.05$) increased relative to controls at all time points except day 3.

Pregnancy—and the consequent elevation in serum hormone concentrations—induced proliferation in the mammary epithelium. In addition, mice treated with 0.05-mg E2 capsules had an increased level of proliferation relative to control animals,

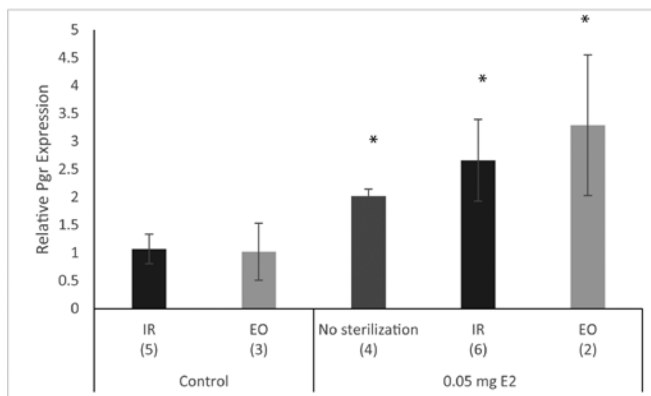

Figure 4. Relative progesterone receptor (*Pgr*) gene expression in the mammary epithelium of mice implanted with silastic capsules. Relative *Pgr* expression is significantly (*, $P < 0.05$) greater in the mammary glands of mice treated with 0.05-mg E2 capsules than in the combined controls, regardless of the sterilization method. The number of animals per group is indicated in parentheses.

according to the percentage of BrdU-positive luminal mammary epithelial nuclei (Figures 5 C and 6 D through F). Proliferation and BrdU incorporation in the mammary glands of untreated control animals was low (positive nuclei, 0.40% ± 0.13%). E2 stimulation significantly increased proliferation, such that 12.9% ± 4.2% of luminal epithelial cells were BrdU-positive by day 3, and this marker remained elevated throughout the 14-d treatment period. In addition, BrdU-positive epithelial nuclei and proliferation were increased on day 3(8.3% ± 3.4%) in mice treated with 0.1 mg E2 and remained elevated throughout the 14-d treatment period. The overall percentage of BrdU-positive cells did not differ between mice treated with 0.05-mg compared with 0.1-mg capsules.

Hematoxylin and eosin staining allowed us to visualize mammary glandular development and alveogenesis after treatment with 0.05 or 0.1 mg E2 for 14 d (Figure 6 A–C). Treatment with E2 induced increased ductal development in contrast to that in untreated controls. With both 0.05- and 0.1-mg E2 capsules, progression through the 14 d of treatment resulted in increased ductal clusters and alveogenesis in the mammary glands of the treated mice.

Discussion

Various methods are available for administering hormones to research animals. We previously have used commercially prepared hormone pellets and have found, similarly to others,[8,19] that these implants yield conspicuously elevated and extended delivery of hormone. The 14-d 0.05-mg pellets we chose elevated serum E2 to supraphysiologic levels that remained elevated even at 56 d after implantation—well beyond the prescribed 14-d time period. Removal of pellets on day 14 resolved the issue of the extended hormone delivery, but these pellets are chalky, difficult to remove, and carry a risk of retained hormone-secreting pellet fragments. In addition, the commercial pellets are marketed to deliver consistent hormone, without 'peaks and valleys.' However, in addition to prolonged supraphysiologic serum E2, we found that serum E2 spiked at day 3. Although our 0.1-mg silastic capsules resulted in a supraphysiologic serum E2 spike (20-fold) at day 3, the interanimal variation with these inhouse-made capsules was more modest than the commercial pellets. Another advantage of the silastic capsule is that estrogen release is easily terminated by removal of the silastic capsule. Because the 0.05-mg commercial pellets secreted greater

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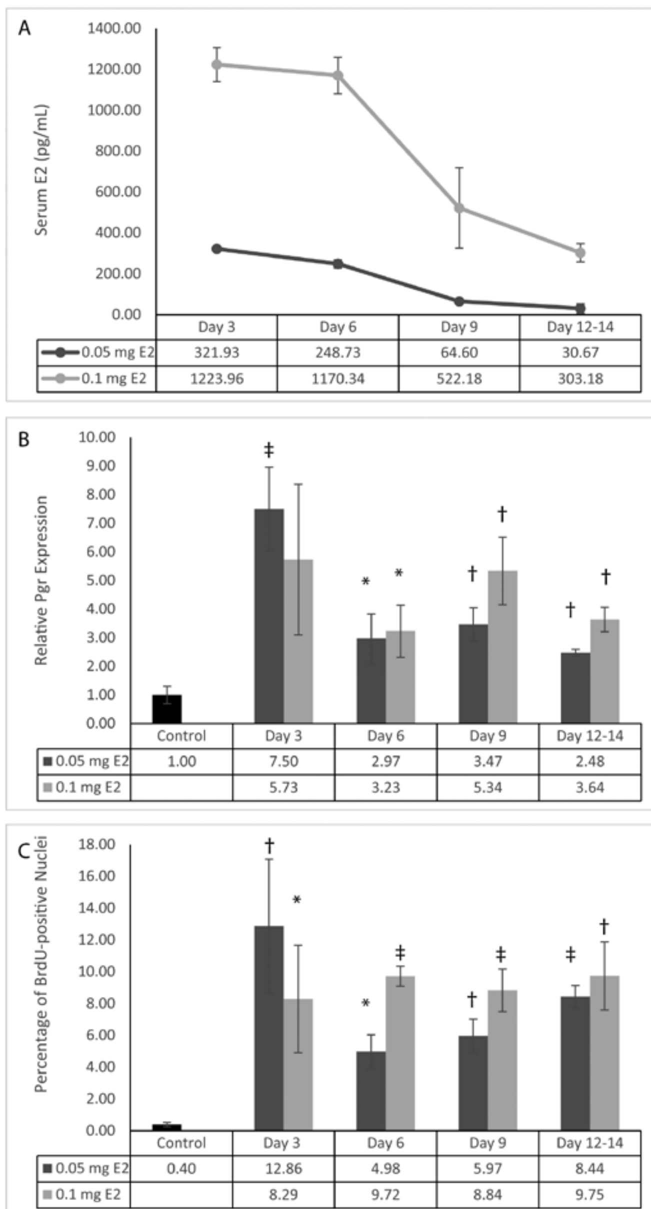

Figure 5. Mammary gland responses to E2 are retained during the treatment period, although serum E2 levels decline beginning 14 d after implantation. (A) Intact female mice were implanted with 5-Gy IR-sterilized silastic capsules containing E2 (0.05 or 0.1mg), and serum E2 levels were determined on days 3, 6, 9 or 12–14 by using a E2-specific ELISA for a subset of 3 mice per time point. (B) Progesterone receptor (Pgr) gene expression and (C) BrdU incorporation are significantly greater than control levels at all time points. *, $P < 0.05$; †, $P < 0.01$; ‡, $P < 0.001$.

concentrations of E2 than the 0.05-mg silastic cellulose capsule and were more similar to those from the 0.1-mg silastic capsule, the matrix provided by commercial pellets might allow more liberal delivery of the hormone, and therefore only requires optimization of the hormone concentration. Optimization of the hormone concentration for the commercial pellets could be resolved by comparing dose curves with various pellet concentrations, as we demonstrated with the 0.05- and 0.1-mg silastic capsules, but the requirement and expense for a minimum order number (25 pellets) does not make this solution feasible. Essentially, in many regards, the commercial pellets and silastic capsules performed similarly, depending on the pellet concentration and produced a spike of supraphysiologic serum E2 with a gradual decline in hormone secretion.

Silastic hormone capsules are routinely used in research settings and provide a simple, cost-effective, and versatile means to administer hormones, such as estrogen, in vivo but require sterilization to satisfy OLAW restrictions. Sterility of implantable devices is typically achieved by either aseptic processing or terminal sterilization.[12,20] E2 is reasonably stable and resistant to radiolysis by γ irradiation at doses between 5 to 26.6 kGy.[16] We demonstrated that 2 common methods for terminal sterilization, γIR and EO, do not alter serum E2 concentrations. Together, the maintenance of serum E2, observed morphologic maturation of the mammary glands, and enhanced *Pgr* expression suggest that either IR or EO can be used to sterilize silastic E2 capsules before implantation, to comply with OLAW regulations without decreasing the systemic activity of the hormone.

We typically administer E2 to both ovariectomized and intact female mice to mimic serum levels achieved through midpregnancy through to parturition.[4] During natural pregnancy, serum E2 concentrations in mice peak at 60 to 110 pg/mL.[15] Therefore, to mimic pregnancy appropriately, serum E2 levels should be sustained above 100 pg/mL for at least 14 d. Sterilization of the silastic E2 capsules by either 5-kGy IR or EO did not impair hormone delivery, and sterilized capsules were not different from nonsterilized E2 capsules in terms of serum E2 concentrations. All 0.5-mg E2 capsules, sterilized or not, resulted in serum E2 levels of about 75 pg/mL on day 14. However, because of the variability of E2 concentrations among mice, we had concerns that not all of the 0.05-mg silastic E2 capsules secreted enough hormone to maintain serum E2 at 100 pg/mL for the entire treatment period. This situation is in contrast to 0.05-mg commercial pellets, which still secreted significantly increased amounts of estrogen after 65 d. Comparison of serum E2 in mice treated with 5-kG IR E2 capsules containing either 0.05 mg or 0.1 mg of hormone showed that the 0.05-mg E2 capsule was sufficient to sustain pregnancy concentrations of serum E2 for only 9 d. The 0.1-mg 5-Gky IR E2 capsule, in contrast, secreted much more hormone than required for the entire 14-d treatment period.

Many factors must be considered when designing hormone delivery products to achieve targeted serum concentrations. In our experiments, 0.05-mg E2 pellets produced much higher serum E2 concentrations on day 3 (approximately 1400 pg/mL) than the 0.05-mg E2-containing cellulose silastic capsules (approximately 300 pg/mL) in BALB/c mice. Likewise, others[8,19] have generated day 2 serum E2 concentrations of 50 to 100 pg/mL by using 0.036-mg E2-containing sesame seed oil silastic capsules in C57BL/6 mice. Hormone concentration, capsule matrix, and capsule surface area all influence hormone release rates. In addition, variation in responses to estrogens among rodent strains should be considered.[11] Although our results show that the serum E2 on day 14 did not differ between nonsterilized capsules and those sterilized with 5-kGy IR, γ irradiation could affect the secretion rates of some capsule materials and matrices.[16]

Our objective with the hormone treatments is to mimic pregnancy-induced changes in the mammary gland. The mammary glands of mature nulliparous mice undergo minimal morphologic development as they cycle through the different phases of the estrus cycle, and the mammary ducts demonstrate simple bifurcation with minimal tertiary branching when viewed as whole mounts.[7] Alternatively, the mammary glands from parous animals or nulliparous animals treated with pregnancy levels of estrogen develop ductal architecture that demonstrates more extensive tertiary branching and alveolar buds. Our 0.05-mg

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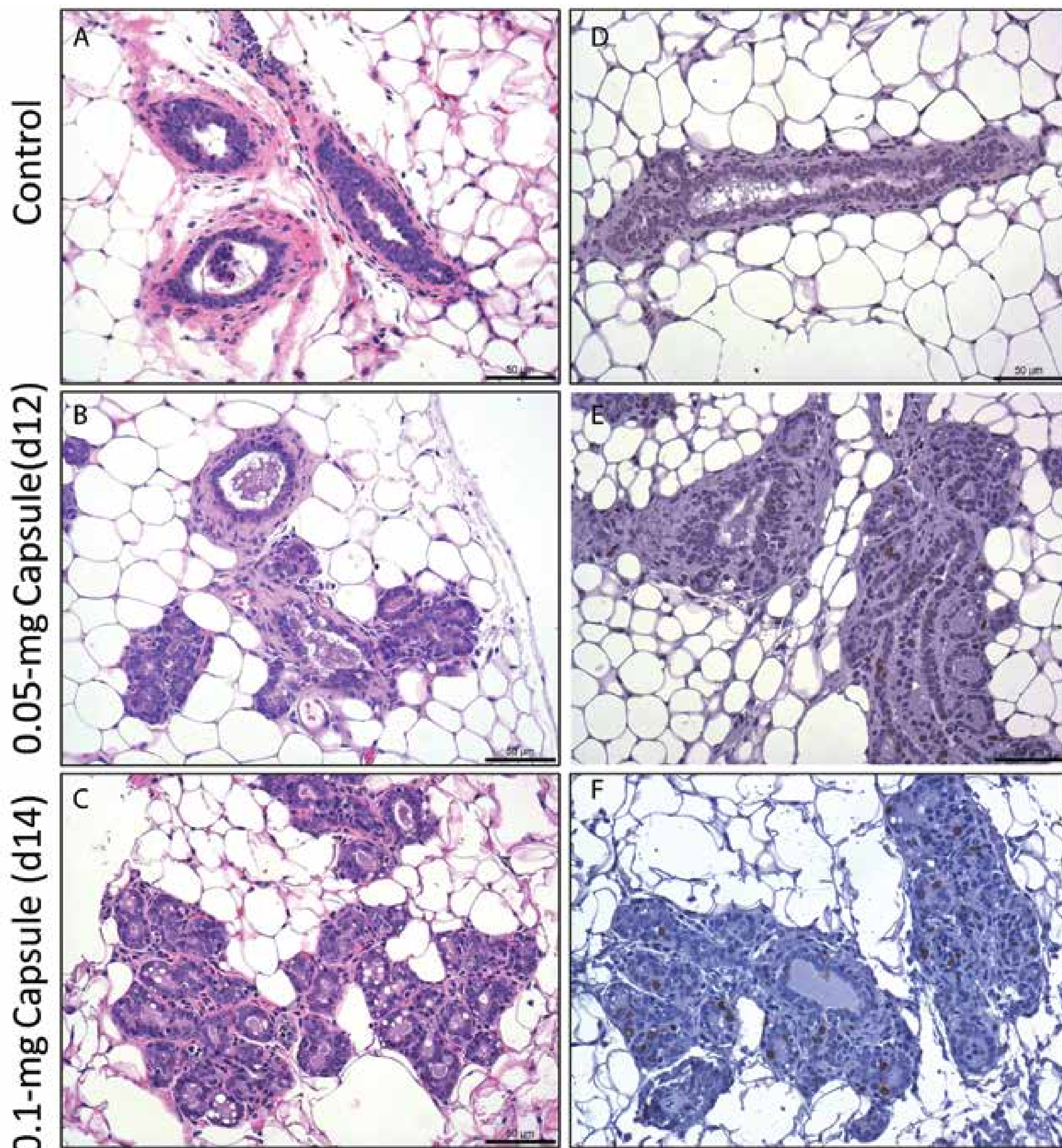

Figure 6. Hematoxylin and eosin staining and BrdU incorporation in mammary glands of mice implanted with silastic capsules. (A–C) Hematoxylin and eosin staining demonstrates differences in ductal structure due to hormone treatment. (A) Control. (B) Day 12, 0.05 mg E2. (C) Day 14, 0.1 mg E2. (D–F) Representative images of 5-bromo-2-deoxyuridine assay to assess cell proliferation among mammary glands of mice implanted with silastic capsules. (D) Control. (E) Day 12, 0.05 mg E2. (F) Day 14, 0.1 mg E2.

silastic E2 capsules, regardless of sterilization, produced the desired effect of tertiary branching and alveogenesis in the mammary glands, which thus were similar to the 14-d pregnant control mammary glands. Similarly, the mammary glands from the mice treated with the sterilized control cellulose capsules resembled nulliparous mammary glands. In terms of an additional marker of appropriate hormone response in the mammary glands, the E2 silastic capsules all induced the expression of progesterone receptor and enhanced proliferation relative to the control cellulose silastic capsules. Therefore, even though 0.05-mg E2 capsules did not sustain serum E2 levels above those necessary to maintain pregnancy, they were sufficient to induce pregnancy-like changes in the mammary glands of treated mice.

In conclusion, hormone silastic capsules are a malleable, convenient method for long-term hormone treatment. We demonstrate that sterilization with 5 kGy ^{137}Cs irradiation or ethylene oxide does not alter E2 secretion from silastic capsules or E2 function in vivo. Either of these terminal sterilization methods can be used to ensure both sterility and efficacy of silastic capsules in animal research models.

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