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Antifertility effectiveness of a novel copper-containing intrauterine device material and its influence on the endometrial environment in rats

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ABSTRACT

This study was designed to investigate the antifertility effectiveness of a novel copper-containing intrauterine device material containing a composite of micro-copper (Cu), low-density polyethylene (LDPE), and methyl vinyl silicone rubber (MVQ) and its effects on the endometrial environment in rats. The contraceptive effectiveness was examined 12 days after pregnancy. The pathological changes; factors associated with bleeding, pain, and inflammation in the endometrium; and the surface condition of the implants were investigated after insertion for 90 days. Furthermore, the release rate of copper ions in simulated uterine solution (SUS) was investigated for 270 days. The contraceptive effectiveness was 100% in both the bulk Cu and micro-Cu/LDPE/MVQ groups, and that in the LDPE/MVQ group was 30%. On day 90 after insertion, histopathological observation and the ultrastructural changes in the endometrium showed that the damage caused by bulk Cu was much more severe than that caused by the Cu/LDPE/MVQ microcomposite and that the surface of the latter was much smoother than that of the former. Furthermore, compared with the sham-operated control group, the concentrations of tissue plasminogen activator and prostaglandin E2 were significantly increased 90 days after insertion in all of the experimental groups except for the LDPE/MVQ group ($P < 0.05$), and the parameters in the Cu/LDPE/MVQ group were significantly lower than those in the Cu group ($P < 0.05$). In addition, the expression levels of matrix metalloproteinase 9, metalloproteinase 1 tissue inhibitor, plasminogen inhibitor 1, CD34, vascular endothelial growth factor, substance P, and substance P receptor in the endometrium in all of the experimental groups were significantly lower than those in the Cu group 90 days after insertion ($P < 0.05$). The results of this study indicate that micro-Cu/LDPE/MVQ exhibits satisfactory contraceptive efficacy and causes fewer side effects than Cu.

1. Introduction

As a reversible, long-acting, safe, and highly effective method of contraception, the copper-bearing intrauterine device (Cu-IUD) is one of the most widely used methods of birth control [1,2]. It is well known that copper ions are necessary for the contraceptive action mechanism of Cu-IUDs. First, the presence of copper ions in the uterine fluid inhibits the activities and capacitation of spermatozoa before fertilization and prevents implantation of the embryo after fertilization [3,4]. Second, the copper ions provoke acute and chronic inflammatory responses within the endometrium; these lead to increased leukocyte infiltration and inflammatory cytokines, which has a spermicidal effect [5].

Although Cu-IUDs have excellent contraceptive efficacy, the existing Cu-IUDs are associated with undesirable side effects, such as menorrhagia, intermenstrual bleeding, pelvic pain, and spotting, that have not yet been overcome in clinical use [6,7] and are important complaints that result in IUD removal [8]. It is believed these disadvantages are likely associated with certain defects in the existing Cu-IUDs, such as the burst release of copper ions within the first months of Cu-IUD insertion [9,10], the increasing coarseness of the surface after long-term corrosion by uterine secretions [11], and direct contact with the endometrium [11]. Although great efforts have been made to improve the structure and to change the size and shape of copper-containing IUDs [12,13], these improvements have not solved these inherent defects.

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To address these defects, polymer matrix composites have been used to reduce the disadvantages of the existing Cu-IUD materials. The polymer composite-based IUDs have the advantage of a controlled release of Cu^{2+} ions [14–17], which could alleviate serious side effects.

Low-density polyethylene (LDPE) and methyl vinyl silicone rubber (MVQ) have been widely used in numerous biomedical applications for their superior biocompatibility and mechanical properties [18,19], Furthermore, MVQ is soft, has excellent flexibility, is not associated with any known toxicity, and is widely used clinically in orthopedic and cardiovascular devices [20]. In our previous research, LDPE was used in polymer matrix composites in IUDs [21], and the polymer matrix composites controlled the release velocity of the copper ion. The antifertility effectiveness, side effects, and safety of the nano-Cu IUDs were extensively studied in mice, rabbits, rats, and rhesus monkeys [21–23]. However, these improvements did not solve the inherent limitations of Cu-IUDs [24].

To overcome or lessen the limitations or shortcomings of the existing Cu-IUDs, we developed a novel Cu-IUD material, a microcomposite of copper, low-density polyethylene, and methyl vinyl silicone rubber (Cu/LDPE/MVQ), and investigated its suitability as a new class of IUD material [25]. Whether the new Cu/LDPE/MVQ microcomposite can meet the needs of a contraceptive device is worthy of consideration. Therefore, we conducted fundamental research to evaluate the antifertility effectiveness of the Cu/LDPE/MVQ microcomposite and its influence on the morphology of the endometrium.

2. Materials and methods

2.1. Preparation of Cu/LDPE/MVQ microcomposite materials

The IUD component materials were prepared in the Department of Materials Science and Engineering of Huazhong University of Science and Technology. The micro-Cu/LDPE/MVQ was constructed using physicochemical methods, combining the MVQ and LDPE powders with 15 wt% high-quality copper microparticles (the whole material weighing about 15 mg) using a melt-blending process in a single-screw extruder at a screw speed of about 20 to 25 rpm. The extruder was maintained at 115 °C, 140 °C, and 155 °C from hopper to die, respectively. The copper microparticles were distributed uniformly in the composites, and the LDPE/MVQ matrix was the frame. The spacing inside offered an osmosis passageway for copper ions and corrosion mediation and could effectively control the corrosion rate by separating the copper microparticles with the matrix; thus, it could control the copper ion release velocity. Three different materials were studied: micro-Cu/LDPE/MVQ, LDPE/MVQ, and bulk copper (Cu). The mean diameter was about 1 mm, and the length was 2 cm. Before the experiment, the material was cut into lengths of 0.78 mm, and the surface area of each sample was 4 mm^2.

2.2. Animals and treatment

Sexually mature female and male Sprague Dawley rats at 8 to 10 weeks of age and weighing 220 to 250 g (female) or 350 to 400 g (male) were purchased from the Experimental Animal Center of Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China. The animals were allowed to acclimatize in an isolated animal room at a constant temperature of 24 °C ± 2 °C and a relative humidity of 50% to 10% with a 12-h light/dark cycle for 1 week before the experiments began. Distilled water and sterilized rat food were provided ad libitum. All protocols for animal care and treatment were approved by the Ethical Committee of Tongji Medical College, Huazhong University of Science and Technology.

One hundred sixty healthy female rats were randomly divided into four groups of 40 each: (a) a sham-operated control group (SO group); (b) the Cu/LDPE/MVQ group; (c) the LDPE/MVQ group; and (d) the Cu group. The rats in the Cu, LDPE/MVQ, and Cu/LDPE/MVQ groups were anesthetized with an intraperitoneal injection of 3% thioethamyl, followed by aseptic performance of laparotomy and uterotomy. The corresponding material was then implanted into the caudal portion of the right uterine horn according to a previously described method [26]. The rats in the SO group underwent the same operations, but nothing was inserted.

Eighty female rats were then mated with male rats at a 1:2 ratio 30 days after IUD insertion. The next morning, vaginal secretions were collected using sterile cotton swabs with saline solution, and the vaginal smears were checked under a microscope. The day on which sperm were found was designated as the first day of pregnancy (D1). The animals were killed on D12, and the pregnancy outcome was observed via uterotomy.

The daily vaginal secretions of the remaining rats were examined to determine the stage of the estrous cycle 90 days after IUD insertion. The rats in the same cycle were then anesthetized with sodium pentobarbital and killed. The endometrial tissues were collected and kept frozen at −80 °C until they were used for experiments. The remaining tissues were fixed for histopathologic examination and transmission electron microscopy (TEM).

2.3. Histopathological and immunohistochemical analysis

To examine the pathological events, the harvested tissue specimens were fixed in 10% neutral-buffered formalin, embedded in paraffin blocks, sliced to 4-μm thickness, and stained with hematoxylin and eosin for observation under a light microscope. Histopathologic evaluation of the sections stained with hematoxylin and eosin was performed to determine the extent of inflammatory cell infiltration.

For immunohistochemical analysis, the slides were incubated with 5% bovine serum albumin (Sigma) for 1 h at room temperature. After rinsing three times in Tris-buffered saline solution (TBS), sections were incubated with the primary antibodies overnight at 4 °C, including tissue inhibitor of metalloproteinase 1 (TIMP1; 1:400; Abcam, #AB61224), matrix metalloproteinase 9 (MMP9; 1:600; Abcam, #AB58803), vascular endothelial growth factor (VEGF; 1:1000; Boster, Wuhan, China, #BA0407), substance P receptor (SPR; 1:2000; Boster, #BA3678), and substance P (SP; 1:100; Abcam, #ab106291). After washing three times in TBS, the slides were incubated with polymerized horseradish peroxidase–conjugated goat anti-rabbit antibody (Tianjin Sungene Biotech Co., Tianjin, China, GB23303) or goat anti-mouse (Beyotime Technology Co., Wuhan, China, #A0216) for 1 h at room temperature and washed three times in TBS. The sections were detected with 3,3′-diaminobenzidine peroxidase substrate, and the nuclei were counterstained with hematoxylin. The slides were examined with an inverted microscope (Olympus Corporation, Tokyo, Japan) at 400× magnification.

2.4. Observation of endometrial ultrastructure using TEM

The endometrium was dissected into 1 × 1-mm pieces and immediately fixed in 2.5% glutaraldehyde in 0.1-M phosphate buffer for 2 h. The samples were then washed three times with 0.1-M phosphate buffer and postfixed for 2 h in 1% osmium in 0.1-M phosphate buffer, rinsed, dehydrated via graded concentrations of ethanol, and embedded using an EMbed 812 kit following a standard procedure. Ultrathin sections from selected blocks were double-stained with uranyl acetate and lead citrate and observed by TEM (JEOL/JEM 1400, Japan).

2.5. Observation of the surface characteristics of the implanted materials using scanning electron microscopy (SEM) and X-ray energy-dispersive spectroscopy (EDS)

The corroded Cu/LDPE/MVQ microcomposite and corroded Cu implants were removed from the rats at day 90 after insertion and used to prepare specimens for observation with SEM and EDS (FEI Quanta