INTRODUCTION

The ideal root canal filling material possesses certain characteristics, such as biocompatibility, adequate marginal sealing quality, dimensional stability, and the ability to allow or induce bone repair and antimicrobial activity. Biocompatibility refers to the ability of a material to perform with an appropriate host response in a specific situation\(^1\). Biocompatibility is one of the factors that influence a clinician’s choice of filling material in root canal treatments as they might be placed in contact with periapical tissues\(^2\). A wide variety of endodontic filling materials are commercially available, including zinc oxide-eugenol cements, glass ionomer cements, epoxy resins, and portland-based cements; but, none meets all the necessary requirements.

AH Plus (Dentsply DeTrey, Konstanz, Germany) is a popular resin-based sealer, although studies have shown seemingly higher cytotoxic effects before AH Plus set\(^3,4\). Another favorite root-end filling material is ProRoot MTA (Dentsply Tulsa Dental, Johnson City, TN, USA), which has good sealing capacity, antimicrobial activity, and biocompatibility\(^5\). However, its drawbacks include difficult handling characteristics, long setting time, and the potential for tooth discoloration associated with the leaching of trace metals from the material\(^6,7\).

Recently, new bioceramic materials were introduced with the intent of duplicating the clinical success of mineral trioxide aggregate (MTA) while improving upon some of its non-ideal properties. iRoot SP (Innovative BioCreamix, Vancouver, Canada) is a convenient, premixed, ready-to-use, injectable, white hydraulic cement paste developed for permanent root canal filling. According to the manufacturer, iRoot SP is an aluminum-free, hydrophilic, calcium silicate-based material that requires the presence of water to set and harden. iRoot SP requires no additional curing agents or mixing, and it delivers a consistent, homogeneous product for filling root canals with or without gutta-percha points. iRoot SP has a composition similar to white mineral trioxide aggregate and has excellent physical properties and antimicrobial activity\(^8,9\).

Biocompatibility of materials is usually evaluated by ex vivo cytotoxicity and in vivo implantation techniques\(^2,10\). To date, few studies have evaluated the subcutaneous and osseous reactions to iRoot SP and MTA. The purpose of this study was to evaluate the reactions of subcutaneous connective tissue and bone tissue to iRoot SP, ProRoot MTA, and AH Plus.

MATERIALS AND METHODS

The protocol of this study was approved by the Ethics Committee of the Hospital of Stomatology, Wuhan University. Thirty-six male Wistar rats, weighing 200 to 250 g, were selected. The rats were housed in temperature-controlled rooms and received water and food ad libitum. The rats were randomly divided into three groups of 12. Each animal underwent a general anesthetic procedure by intraperitoneal injection of 10% ketamine chloride solution (25 mg/kg) and xylazine (10 mg/kg).

Subcutaneous implant test

The dorsal skin of rats was shaved and disinfected with 5% iodine solution. Four 1-cm-long incisions, 2 cm apart from each other, were made on the back of rats along the spine. Two incisions were made on the right half,
and the other two on the left half, of the dorsal skin. Sterile polyethylene tubes (1.5 mm inner diameter, 7 mm length), filled with iRoot SP, ProRoot MTA, or AH Plus cement, were prepared according to manufacturers’ instructions under aseptic conditions. Each animal received four polyethylene tubes. Three test materials were implanted into three separate incisions created by blunt dissection. The fourth incision received an empty tube as a control in each rats. All incisions were closed with 3-0 silk sutures.

**Intraosseous implant test**
The tibia incision area was shaved and the skin disinfected with 5% iodine solution. Under sterile conditions, a longitudinal incision of 30 mm was made at the lateral aspect of the anterior border of tibia reaching the bone. Implantation cavities were prepared in an area of the diaphyseal bone with a diameter and depth of 1 mm, using a round carbide bur (Dentsply Maillefer, Ballaigues, Switzerland) in a low-speed handpiece under normal saline irrigation. Four cavities were prepared at least 1 cm from each other in each animal.

iRoot SP, ProRoot MTA, and AH Plus were prepared according to manufacturers’ recommendations and directly placed in the osseous cavities. Cavities for negative control were prepared in the same manner, but no materials were placed in them. Incisions were closed with 3-0 silk sutures, and the animals were subject to the same dietary and environmental conditions.

**Evaluation parameters**
The animals were divided into three groups of 12 with respect to experimental duration. At the end of each experimental period of 7, 30, and 60 days, rats of respective groups were sacrificed by anesthetic overdose. The tubes and surrounding tissues were excised and fixed in 10% buffered formalin solution for 24 h. Sections of 5 μm thickness were made perpendicular to and as near as possible to the opening of tubes and stained with hematoxylin and eosin to evaluate inflammatory reactions. Animals’ tibias were removed and placed in 10% buffered formalin solution for 24 h. Sections were examined and evaluated in a blind manner under a light microscope (Carl Zeiss, Oberkochen, Germany) at ×100, ×200, and ×400 magnifications by an observer blind to all the procedures involved. Quantitative evaluations of inflammatory cells (lymphocytes, plasmacytes, neutrophils, giant cells, and macrophages) were done in 10 separate areas of each specimen at ×400 magnification. An average of value for each material was obtained from 10 separate areas. Reactions in the subcutaneous and intraosseous tissues in contact with the materials were scored according to previous studies as: Grade 0, none or few inflammatory cells and no reaction; Grade 1, less than 25 cells and mild reaction; Grade 2, between 25 and 125 cells and moderate reaction; Grade 3, 125 cells and more cells and severe reaction. Tissue necrosis was categorized as absent or present. Average thickness of the fibrous capsules of each specimen was evaluated in 10 separate areas. Fibrous capsules were considered to be thin when thickness was <150 μm and thick at >150 μm.

Evaluation of new bone formation around the implanted materials was carried out as follows: Grade 0, no bone formation; Grade I, Slight, presence of bony islets and coverage of less than 25% of the material surface with bone; Grade II, Moderate, coverage of at least 50% of the material surface with bone; Grade III, Extensive, complete coverage of the material surface with bone or the formation of an osseous bridge around the material. Results were statistically analyzed by Kruskal-Wallis and Mann-Whitney tests. Significance was established at p<0.05.

**RESULTS**

**Evaluation of inflammation**

1. Specimens evaluated after 7 days
In subcutaneous implant test, all groups presented Grade 2 inflammation (moderate infiltration of chronic inflammatory cells). No differences were observed among the groups (p>0.05) (Table 1 and Fig. 1).

In intraosseous implant test, moderate to severe infiltration of inflammatory cells was observed in the three experimental and control groups. No significant differences were observed among the groups (p>0.05) (Table 1).

2. Specimens evaluated after 30 days
In subcutaneous implant test, there were statistically significant differences in inflammatory cell number among the four groups (p<0.05). AH Plus group presented Grade 2 inflammation and showed more infiltration of inflammatory cells (lymphocytes and plasmacytes) than the other three groups (p<0.05) (Table 1 and Fig. 2). iRoot SP, ProRoot MTA, and control groups presented Grade 1–2 inflammation (mild to moderate infiltration of chronic inflammatory cells). There were no statistically significant differences among iRoot SP, ProRoot MTA, and control groups (p>0.05) (Table 1 and Fig. 2).

In intraosseous implant test, the number of inflammatory cells decreased in all the four groups. Inflammatory reactions were graded as 1 or 2 (Table 1) with no statistically significant differences among the groups (p>0.05).

3. Specimens evaluated after 60 days
All groups presented Grade 0–1 inflammation. No differences were observed among the groups (p>0.05) (Table 1 and Fig. 3). iRoot SP, ProRoot MTA, and control groups showed thin fibrous capsules, while AH Plus group had thick fibrous capsules (Table 1 and Fig. 3).

In intraosseous implant test, inflammatory reactions were graded as 1 or 0 (Table 1, Figs. 4 and
Table 1  Presence of connective tissue necrotic areas and inflammation grades for each period and group in subcutaneous and intraosseous implant tests

<table>
<thead>
<tr>
<th>Implant material</th>
<th>Connective tissue necrotic areas</th>
<th>Inflammatory grades in subcutaneous implant test</th>
<th>Inflammatory grades in intraosseous implant test</th>
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<td>Control</td>
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<td>AH Plus</td>
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<td>0</td>
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* Indicates a statistically significant difference between the groups ($p<0.05$).

5) with no statistically significant differences among all the groups ($p>0.05$).

**Evaluation of bone formation**

Bone formation processes were similar in iRoot SP, MTA, and control groups, with no statistically significant differences ($p>0.05$) (Figs. 4 and 5). Bone formation results around the experimental materials after 7, 30, and 60 days were Grade I, II, and III respectively. With time, new bone was formed in the control group and grew into the bone defect region. On 30th day, bony islets were observed and surface coverage of iRoot SP was at least...
Fig. 2  Subcutaneous tissue reactions on 30th day.
(a) iRoot SP: Mild to moderate inflammatory reaction (Grade 1–2). Necrotic tissue areas were observed with material particles in contact with the connective tissue (black arrow). Presence of multinucleated giant cells associated with areas of tissue necrosis (red arrow); (b) ProRoot MTA: Grade 1–2 inflammation; (c) AH Plus: Grade 2 inflammation with more infiltration of inflammatory cells (lymphocytes and plasmacytes) than the other three groups; (d) Control: Grade 1–2 inflammation.

Fig. 3  Subcutaneous tissue reactions on 60th day.
(a) iRoot SP: Grade 0–1 inflammation with thin fibrous capsule formation (black arrow); (b) ProRoot MTA: Grade 0–1 inflammation with thin fibrous capsule formation (black arrow); (c) AH Plus: Grade 0–1 inflammation with thick fibrous capsule formation (red arrow); (d) Control: Grade 0–1 inflammation with thin fibrous capsule formation (black arrow).
Fig. 4  Tissue reactions in intraosseous implant test.
(a) iRoot SP group on 7th day: Grade 2–3 inflammation; (b) iRoot SP group on 30th day: Grade 1–2 inflammation and bone bridge were observed, and surface coverage of iRoot SP was at least 50% (arrow); (c) iRoot SP group on 60th day: Grade 0–1 inflammation, complete bone bridge formation on the surface of iRoot SP and grew into the material’s gaps. (arrow); (d) ProRoot MTA group on 7th day: Grade 2–3 inflammation; (e) ProRoot MTA group on 30th day: Grade 1–2 inflammation, new bone formed; (f) ProRoot MTA group on 60th day: Grade 0–1 inflammation, new bone formed in direct contact with ProRoot MTA.

Fig. 5  Tissue reactions in intraosseous implant test.
(a) AH Plus group on 7th day: Grade 2–3 inflammation; (b) AH Plus group on 30th day: Grade 1–2 inflammation, new bone formed; (c) AH Plus group on 60th day: Grade 0–1 inflammation, new bone formed in direct contact with AH Plus; (d) Control group on 7th day: Grade 2 inflammation; (e) Control group on 30th day: Grade 1 inflammation, new bone formed; (f) Control group on 60th day: Grade 0–1 inflammation, new bone formed and grew into the bone defect region.

50%. On 60th day, newly formed bone completely covered the surface of iRoot SP and grew into the material’s gaps (Fig. 4). For ProRoot MTA and AH Plus on 60th day, new bone was formed in direct contact with these materials but not observed inside the materials.
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DISCUSSION

Endodontic materials might affect periradicular tissues either by direct contact or by leached components that are released into the surrounding soft and hard tissues through dentinal tubules, accessory or lateral canals, and apical foramina. Therefore, an endodontic material must be biocompatible and have the ability to allow or induce bone repair\(^{19}\). Implantation experiments in which materials come in direct contact with subcutaneous or osseous tissues have been widely accepted as methods to evaluate their biocompatibility\(^{15,16}\). Implantation periods used in this study were consistent with the majority of studies carried out on tissue reactions to materials implanted in subcutaneous connective tissue or bone tissue\(^{14,17}\).

In the present study, all the materials had a high inflammation grade on 7th day. This was consistent with the results of previous studies\(^{12,13}\). Trauma from inflammation grade on 7th day. This was consistent similar to that previously reported\(^{23,24}\). Results on 7th day showed a moderate inflammatory reaction that decreased on 30th day, and which significantly decreased by 60th day. Thin capsules surrounding the tube were also observed. ProRoot MTA contained calcium oxide after setting, which formed calcium hydroxide with tissue fluids. The resultant calcium hydroxide might contribute to the early biocompatibility of the material\(^{20}\). In a study by Sarkar et al., MTA exposed to synthetic tissue fluid produced precipitates which were similar to hydroxypatite layer\(^{20}\). Hydroxypatite layers, which have low toxicity, are highly biocompatible. This layer might have osteogenic potential because it can release calcium and phosphorus ions, which are involved in bone metabolism. This property might have a role in the biocompatibility of MTA and iRoot SP. The present study showed that ProRoot MTA and iRoot SP had similar tissue responses to the formation of hard tissues.

In the present study, AH Plus sealer showed a moderate inflammatory reaction on 30th day. The mechanism that might account for this inflammatory response was the release of formaldehyde, which has been shown to induce non-neoplastic responses such as epithelial degeneration and a mixed inflammatory cell infiltration, besides allergic reactions and necrosis of connective tissues\(^{27}\). Amines—present in AH Plus—accelerate polymerization and could be also the reason for such strong initial response\(^{24}\). Furthermore, inflammatory activity together with blood supply in the tissue repair process could eliminate initial toxicity of the material\(^{20}\).

Based on the results of the present in vivo study, it was concluded that iRoot SP had favorable properties with respect to the biological responses of subcutaneous and bone tissues. iRoot SP seemed to be a promising root canal cement for widespread use. Our research showed that biological response to iRoot SP was similar to that of ProRoot MTA, but further studies are required.

ACKNOWLEDGMENTS

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