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# Cell migration and osteo/odontogenesis stimulation of iRoot FS as a potential apical barrier material in apexification

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## Abstract

**Liu Y, Liu XM, Bi J, Yu S, Yang N, Song B, Chen X.** Cell migration and osteo/odontogenesis stimulation of iRoot FS as a potential apical barrier material in apexification. *International Endodontic Journal*, **53**, 467–477, 2020.

**Aim** To investigate the *in vitro* biological effects of a nanoparticle bioceramic material, iRoot Fast Set root repair material (iRoot FS), on the proliferation, migration and osteo/odontogenic differentiation of human stem cells from the apical papilla (hSCAP), and to further explore the mechanism involved in osteo/odontogenic induction of iRoot FS.

**Methodology** hSCAP were isolated and characterized *in vitro*. iRoot FS conditioned medium were prepared and used to treat hSCAP, while using mineral trioxide aggregate (MTA) conditioned medium as the positive control and regular medium as the negative control. MTT assay and BrdU labelling assay were performed to determine cell proliferation. Wound healing assay and transwell assay were conducted to evaluate cell migration. The osteo/odontogenic differentiation of hSCAP was evaluated by qPCR, Western blot and Alizarin red S staining. Wnt inhibitor was used for downregulating the expression level of β-catenin of hSCAP.

**Results** The cell proliferation of hSACP in the iRoot FS group was not significantly different compared with the control groups. The cell migration of hSCAP in the iRoot FS group was significantly increased than the MTA and negative control groups ($P < 0.01$). The expression levels of osteo/odontogenic markers and mineralization nodule formation of hSCAP in the iRoot FS group were significantly elevated ($P < 0.01$). Furthermore, iRoot FS enhanced the osteo/odontogenic differentiation of hSCAP by activating Wnt/β-catenin signalling.

**Conclusions** iRoot FS promoted the cell migration of hSCAP and enhanced their oseto/odontogenesis potential *via* the Wnt/β-catenin pathway without cytotoxicity. iRoot FS had satisfactory biological properties and has potential to be used as an apical barrier in apexification or as a coronal sealing material in regenerative endodontic treatment.

**Keywords:** apexification, cell migration, cell proliferation, nanoparticle bioceramic material, osteo/odontogenic differentiation, stem cells from apical papilla.

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## Introduction

Calcium hydroxide (CH) is commonly used to induce apical closure after disinfection of the root canal during apexification. However, apexification with CH has several limitations including unpredictable continued root closure, the risk of a re-infection and root fracture (Andreasen *et al.* 2002).

An alternative to CH therapy is placement of an apical plug or barrier. Due to its excellent sealing properties and biocompatibility, mineral trioxide aggregate (MTA) has been used as an apical plug to

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induce the formation of an apical calcified barrier (Holden *et al.* 2008, Duggal *et al.* 2017). However, MTA has several limitations such as long setting time, grainy consistency and is associated with tooth discolouration (Parirokh & Torabinejad 2010, Shi *et al.* 2016). During apexification, it is difficult to handle and place MTA in the root-end, which can weaken the mechanical properties of the apical plug and increase the risk of contamination (Kang *et al.* 2013). In addition, the lack of continued apical development reduces the therapeutic outcomes of the apical plug technique (Torabinejad *et al.* 2017). Therefore, a short setting time and simplified operation without discolouration and excellent inductive capacity of root development are relevant characteristics for the ideal apical barrier material in apexification.

iRoot Fast Set root repair material (iRoot FS) (Innovative BioCeramix, Vancouver, BC, Canada) is a nanoparticle bioceramic material composed of calcium silicates, zirconium oxide, tantalum pentoxide, calcium phosphate monobasic, anhydrous calcium sulphate and filler agents (Yang & Lu 2008). iRoot FS has a short setting time and is made ready-to-use. It has been used as a permanent root canal repair material in endodontic treatments and apical surgery. Recent studies reported that iRoot FS set completely within 1 h, while MTA setting required at least 5 to 7 days (Jiang *et al.* 2014). iRoot FS also has similar mechanical properties and biocompatibility to MTA (Lv *et al.* 2017). Sun *et al.* (2017) examined the effects of iRoot FS on the odontogenic differentiation of human dental pulp stem cells, which suggested that iRoot FS was a promising bioactive material for direct pulp capping. Luo *et al.* (2018) investigated the cytotoxicity and biocompatibility of iRoot FS with human periodontal ligament cells and found that iRoot FS could be used as a root-end filling material in endodontic surgery. However, it is still unknown whether iRoot FS can induce apical closure of immature permanent teeth, and whether it is suitable as an apical barrier during apexification.

Stem cells from the apical papilla (SCAP) are a unique population of highly proliferative neural crest-derived mesenchymal stem cells isolated (Sonoyama *et al.* 2008). Since SCAP may survive and maintain their stemness in necrotic pulpitis and apical periodontitis of immature permanent teeth, they have been identified as the most important cell source for pulp-dentine tissue and continued root development (Sonoyama *et al.* 2006, 2008). Therefore, the biological effects of an apical barrier material on SCAP are critical to therapeutic outcomes of apexification. However, there are no currently reports on cellular responses for SCAP on iRoot FS. In this study, the effects of iRoot FS on cell proliferation, cell migration and osteo/odontogenic differentiation of human SCAP (hSCAP) were evaluated, and the molecular mechanism involved in osteo/odontogenic potential of iRoot FS was further explored.

## Materials and methods

### Isolation and culture of hSCAP

The study was approved by the Ethics Committee of the School of Stomatology, China Medical University (201315). Human impacted third molars with immature roots were obtained from healthy patients (under 18 years old) at the School of Stomatology affiliated with the China Medical University. The apical papilla was separated gently, then digested in a solution of 3 mg $mL^{-1}$ collagenase type I (Worthington Biochemical Co., Lakewood, CO, USA) and 4 mg $mL^{-1}$ dispase II (Boehringer Ingelheim, Mannheim, Germany) for 1 h at 37°C. Single-cell suspensions were cultured in a regular medium in a humidified atmosphere at 37°C (5% $CO_2$). Three to 5 passage cells were harvested and used in further experiments. The detailed protocol has been reported previously, and hSCAP were characterized with $CD73^+/CD90^+/CD105^+/CD146^+/CD31^-/CD34^-$ immune-phenotypes (Bi *et al.* 2018). According to methods used in previous studies (Sonoyama *et al.* 2006, Aquilar & Lertchirakarn, 2016), the unique marker CD24 expression in hSCAP (data not shown) was identified in this study.

### Preparation of iRoot FS and MTA conditioned medium

iRoot FS and ProRoot MTA (Dentsply Tulsa Dental, Tulas, OK, USA) were evaluated. The materials were prepared according to the manufacturer's instructions under sterile conditions. According to previous studies (Luo *et al.* 2014, Chen *et al.* 2016, Sun *et al.* 2017), iRoot FS and MTA were prepared at the concentration of 2 mg $mL^{-1}$ that has been demonstrated to improve cell viability and differentiation potential.

As an apical barrier in apexification, the released inorganic ions from iRoot FS and MTA play important roles for the outcomes of clinical treatment.

Moreover, iRoot FS and MTA could dissolve in culture medium in the tests for *in vitro* cytotoxicity; therefore, the material extractions were used to evaluate the cytotoxicity on hSCAP. According to ISO 10993, the liquid extracts of materials for biological evaluation of iRoot FS were prepared. The 0.2 g materials were placed into polyethylene moulds with a diameter of 10 mm and thickness of 1 mm to achieve an appropriate mass/volume of extraction. The material samples were stored in humid conditions at 37°C for complete setting, and then the moulds were removed. In order to obtain the maximal dissolution, each material sample was incubated in 1 mL alpha modification of Eagle's medium (α-MEM, HyClone, UT, USA) for 7 days at 37°C. The extraction was passed through a 0.22 μm filter. For 2 mg $mL^{-1}$ of conditioned medium, the extraction was diluted with regular medium containing α-MEM, supplemented with 15% FBS (ExCell Bio, Shanghai, China), 100 mmol $L^{-1}$ L-2 ascorbic acid phosphate (Sigma-Aldrich, St. Louis, MO, USA) and 100 U $mL^{-1}$ penicillin/streptomycin (HyClone).

### BrdU labelling assay

hSCAP ($1 \times 10^4$ cells/well) were seeded on 2-well chamber slides (Nunc, Rochester, NY, USA). The cultures were incubated in BrdU solution (1: 100) (Invitrogen, NY, USA) for 20 h and stained with a BrdU staining kit (Invitrogen) according to the manufacturer's instructions. The samples were then stained with haematoxylin. BrdU-positive cells and total cell numbers were counted on five images per specimen. The number of BrdU-positive cells was represented as a percentage of the total cell number. The BrdU labelling assay was repeated on three independent samples for each experimental group, and the average percentage of BrdU-positive cells was used.

### Methylthiazolyldiphenyl-tetrazolium bromide (MTT) assay

hSCAP ($5 \times 10^3$ cells/well) were seeded on 96-well plates and treated with conditioned medium for 3 days. The proliferation rate was evaluated using the MTT assay at 1, 2, 3, 4 days. Briefly, 10 μL of the 12 mmol $L^{-1}$ MTT stock solution (Life Technologies, NY, USA) was added to each well, and the plates were incubated for 4 h at 37°C. The precipitate was extracted with DMSO (Sigma-Aldrich), and the optical density was measured at a wavelength of 540 nm.

### Wound healing assay

hSCAP ($1 \times 10^4$ cells/well) were seeded on 12-well plates until 70-80% confluence. A scratch was gently and slowly made by using a 1 mL pipette tip across the centre of the well. hSCAP were treated with conditioned medium for 12 and 24 h, respectively. The cells were washed with PBS, fixed with 3.7% paraformaldehyde for 30 min. The gap distance was quantitatively evaluated on five images per specimen using Image J software. The wound healing assay was repeated on three independent samples for each experimental group. The mean value of gap distance was used in the analysis.

### Transwell migration assay

A cell migration transwell system (12 wells/plate) was used. hSCAP ($1 \times 10^5$ cells/well) were seeded on the upper chamber, and 2 mL of conditioned medium was added into the lower chamber. After 24 h, the cells under the membrane were stained with 1% toluidine blue solution. The number of cell in each sample was determined by counting 5 images for each specimen. The cell migration assay was repeated on three independent samples for each experimental group. The mean value of stained cell numbers was used.

### *In vitro* osteo/odontogenic differentiation assay

hSCAP were cultured in osteo/odontogenic medium containing 1.8 mmol $L^{-1}$ monopotassium phosphate (Sigma-Aldrich) and 10 nmol $L^{-1}$ dexamethasone (Sigma-Aldrich). After 6 days of induction, the cells were extracted for RNA isolation, and then qRT-PCR was used to detect the expression levels of osteo/odontogenic genes. Ten days after induction, the total protein of the cells was lysed and Western blot was used to detect the expression levels of osteo/odontogenic proteins. After 4 weeks of induction, the culture cells were stained with Alizarin red S to detect mineralized nodule formation, and the stained area was evaluated quantitatively.

### Quantitative real-time reverse-transcriptase polymerase chain reaction (qRT-PCR)

qRT-PCR was performed using GoTaq® qPCR Master Mix (Promega Corporation, Madison, WI, USA) in a 7500 real-time PCR system (Applied Biosystems). The mRNA expression levels of dentine sialophosphoprotein

(*DSPP*) and alkaline phosphatase (*ALP*) were evaluated. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as a housekeeping gene for normalization. The sequences of primers were as follows:

*DSPP* forward primer, 5′-CTGTTGGGAAGAGCCAAGATAAG-3′;

*DSPP* reverse primer, 5′-CCAAGATCATTCCATGTTGTCCT-3′;

*ALP* forward primer, 5′-TAAGGACATCGCCTACCAGCTC-3′;

*ALP* reverse primer, 5′-TCTTCCAGGTGTCAACGAGGT-3′;

*GAPDH* forward primer, 5′-CCGGCGTCCGACCTGTGAAC-3′;

*GAPDH* reverse primer, 5′-GGGCGAAGGCTCCAGAGGA-3′.

### Western blot analysis

Total protein was extracted using M-PER mammalian protein extraction reagent (Thermo, Rockford, IL, USA). Twenty μg of protein were applied and separated on 4–12% NuPAGE gel (Invitrogen), followed by transferring to nitrocellulose membranes (Millipore, Billerica, MA, USA). Membranes were blocked with 5% nonfat dry milk and 0.1% Tween-20 for 1 h, followed by incubation with the primary antibodies DSPP (Santa Cruz Biotechnology, Santa Cruz, CA, USA; 1: 200), ALP (1: 200, Santa Cruz Biotechnology), nuclear β-catenin (1: 1000, Millipore) and total β-catenin (1: 1000, Millipore) at 4°C overnight. Horseradish peroxidase (HRP)-conjugated secondary antibody (Santa Cruz Biotechnology; 1: 10 000) was used to treat the membranes for 1 h. Immunoreactive proteins were detected using SuperSignal West Pico Chemiluminescent Substrate (Thermo) and BioMax film (Kodak, Rochester, NY, USA). Each membrane was also stripped using a stripping buffer (Thermo) and re-probed with β-actin antibody to quantify the amount of loaded protein.

### Alizarin red S staining

After 4 weeks of induction, the cultures were fixed with 60% isopropanol and stained with Alizarin red S for mineralized nodule formation. Nonspecific staining was removed by repeated washing with distilled water. The formation of mineralized nodules was photographed using a stereoscopic microscope. The stained area in each well was evaluated quantitatively in 5 images per specimen using Image J software. The Alizarin red S staining was repeated on three independent samples for each experimental group.

### Wnt/β-catenin signalling assay

hSCAP ($1 \times 10^5$ cells/well) were seeded on 6-well plates and treated with conditioned medium for 3 days. Then, the 20 μmol $L^{-1}$ Wnt/β-catenin inhibitor (XAV939, Sigma-Aldrich) was added in culture system for 24 h. The cell protein was lysed for further experiment to evaluate the Wnt/β-catenin expression by Western blot. The Anti-Active-β-catenin antibody (05-665, Millipore) was used to detect nuclear β-catenin, and the Anti-β-catenin antibody (06-734, Millipore) was used to detect both cytoplasmic β-catenin and nuclear β-catenin.

### Statistical analysis

SPSS 22.0 software (SPSS Inc., Chicago, IL, USA) was used to perform statistical analysis. All data were expressed as mean ± standard deviation (SD). Comparisons between more than two groups were analysed using one-way analysis of variance (ANOVA), and comparisons between each of two groups were analyed by independent two-tailed Student's *t*-test. *P* values <0.05 were considered statistically significant.

## Results

### iRoot FS had no effects on hSCAP proliferation

To evaluate the effects of iRoot FS on the cell properties of hSCAP, the iRoot FS and MTA conditioned medium for the experiments was prepared with regular medium being used as the control group (Fig. 1a). First, the pH values of those three media were tested. The pH value of MTA conditioned medium was 8.823 ± 0.078, the pH value of iRoot FS conditioned medium was 8.837 ± 0.060 and that of the control medium 7.823 ± 0.136 (Fig. 1b). The pH values of iRoot FS conditioned medium were significantly higher than that of the control group ($P < 0.001$), while there was no significant difference in the pH values between iRoot FS and MTA groups ($P = 0.826$) (Fig. 1b).

To identify whether iRoot FS affected hSCAP proliferation, the proliferation rate of hSCAP was examined using the BrdU labelling and MTT assays. The BrdU-positive cell ratio of hSCAP cultured in iRoot FS

 

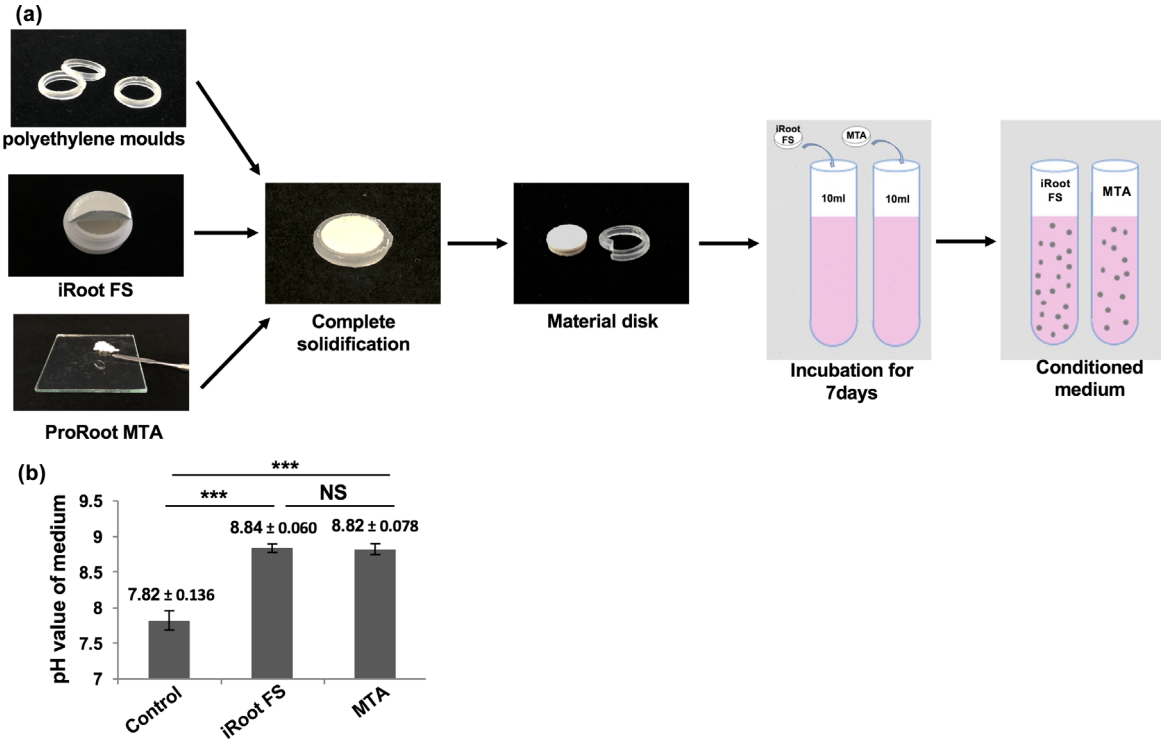


**Figure 1** Preparation of iRoot FS and MTA conditioned medium. (a) The materials were placed into polyethylene moulds (diameter of 10 mm and thickness of 1 mm), respectively, and incubated in 37°C for complete solidification, and then the moulds were taken out. For conditioned medium, each material sample was incubated in 37°C for 7 days in 10 mL of regular medium. (b) The pH value of iRoot FS conditioned medium was higher than that of the control group (***$P < 0.005$), while there was no significant difference in the pH value between iRoot FS group and MTA group. NS = no significant difference, Error bars: means ± SD

conditioned medium was not significantly different when compared with the MTA group ($P = 0.345$) or control group ($P = 0.577$) (Fig. 2a,b). Furthermore, there was no significant difference in proliferation rate of cultured hSCAP in day 1, 2, 3, 4 among the iRoot FS, MTA ($P = 0.054$) and control groups ($P = 0.062$) (Fig. 2c). These data indicated that iRoot FS had no effects on hSCAP proliferation.

### iRoot FS improved the cell migration of hSCAP

The effect of iRoot FS on the cell migration of hSCAP was explored by wound healing and the transwell cell migration assay. The wound closure percentage of hSCAP cultured in iRoot FS conditioned medium was significantly decreased at 12 and 24 h, compared to the MTA ($P = 0.043$ for 12 h, $P = 0.004$ for 24 h) and control groups ($P = 0.003$ for 12 h, $P = 0.004$ for 24 h) (Fig. 3a,b). The number of migrated cells in the iRoot FS group was significantly higher than the MTA ($P = 0.006$) group and control group ($P < 0.001$), as assessed by the transwell assay (Fig. 3c,d). Meanwhile, cell migration of hSCAP in MTA group was significantly greater compared with the control group, as indicated by the decreased wound closure ($P = 0.047$ for 24 h) and the increased number of migrated cells ($P = 0.01$) (Fig. 3a–d). These data demonstrated that iRoot FS had markedly stronger capacity for enhancing cell migration of hSCAP compared with MTA.

### iRoot FS enhanced the osteo/odontogenic differentiation of hSCAP *via* Wnt signalling

To identify the influence of iRoot FS on the osteo/odontogenic differentiation of hSCAP, hSCAP were pretreated with iRoot FS, MTA and control media, respectively, and then cultured in osteo-inductive conditions. Alizarin red S staining revealed that both iRoot FS and MTA significantly increased mineralized

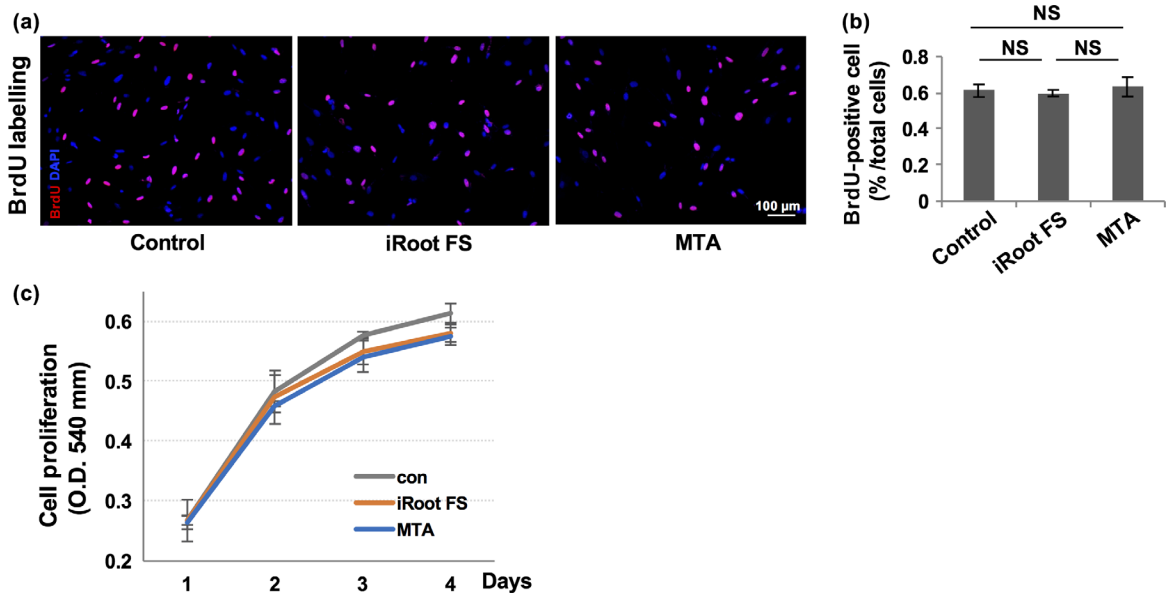


**Figure 2** iRoot FS had no effects on hSCAP proliferation. (a,b) BrdU labelling assay showed that the BrdU-positive cell ratio of hSCAP cultured in iRoot FS conditioned medium had no significant changes when compared with the MTA and control groups. (c) MTT assay showed that iRoot FS had no significant effects on the cell proliferation rate of hSCAP compared to the MTA and control groups. NS = no significant difference, Error bars: means ± SD

nodule formation compared with the control group ($P = 0.001$ for iRoot FS, $P = 0.002$ for MTA), and iRoot FS had significantly stronger capacity for improving mineralized nodule formation ($P = 0.032$) (Fig. 4a,b). Moreover, hSCAP pretreated by iRoot FS had significantly elevated expression levels of *DSPP* ($P = 0.002$ for MTA, $P < 0.001$ for control) and *ALP* ($P = 0.002$ for MTA, $P < 0.001$ for control), as assessed by qPCR analysis (Fig. 4c,d). Western blot analysis also confirmed that iRoot FS improved the osteo/odontogenic differentiation of hSCAP when compared with MTA and control groups, as indicated by the increased protein levels of DSPP and ALP (Fig. 4e). Collectively, these data demonstrated that iRoot FS significantly enhanced the osteo/odontogenic differentiation of hSCAP, even more than MTA.

To clarify the molecular mechanism of iRoot FS treatment in hSCAP, the osteo/odontogenesis relative signalling pathway was explored, including the Wnt/β-catenin and BMP/Smad pathways. iRoot FS significantly activated the Wnt/β-catenin pathway, but not the BMP/Smad pathway (Figure S1). Then, the role of Wnt signalling in iRoot FS mediated osteo/odontogenic differentiation was investigated. Western blot revealed that the expression level of the nuclear β-catenin (nonphosphorylated), but not the total β-catenin (both of cytoplasmic β-catenin and nuclear β-catenin), was markedly increased in iRoot FS-treated hSCAP; however, the Wnt inhibitor (XAV939) inhibited the elevated expression level of nuclear β-catenin (Fig. 5a). XAV939 treatment blocked iRoot FS-induced elevation of SCAP osteo/odontogenesis, as indicated by significantly decreased mineralized nodule formation ($P = 0.005$) (Fig. 5b,c). XAV939 treatment also significantly reduced the gene expression levels of osteo/odontogenic markers of iRoot FS-treated hSCAP, including *DSPP* ($P < 0.001$) and *ALP* ($P = 0.001$) (Fig. 5d,e). Western blot analysis also revealed that XAV939 treatment significantly decreased the protein levels of DSPP and ALP of iRoot FS-treated hSCAP (Fig. 5f). These results indicated that iRoot FS enhanced the osteo/odontogenic differentiation of hSCAP by activating Wnt/β-catenin signalling.

## Discussion

Materials used for apical barriers play a critical role in sealing the apical root canal during apexification and should ideally be able to actively stimulate mineralized tissue formation and continued root development. SCAP are isolated from the apical papilla and

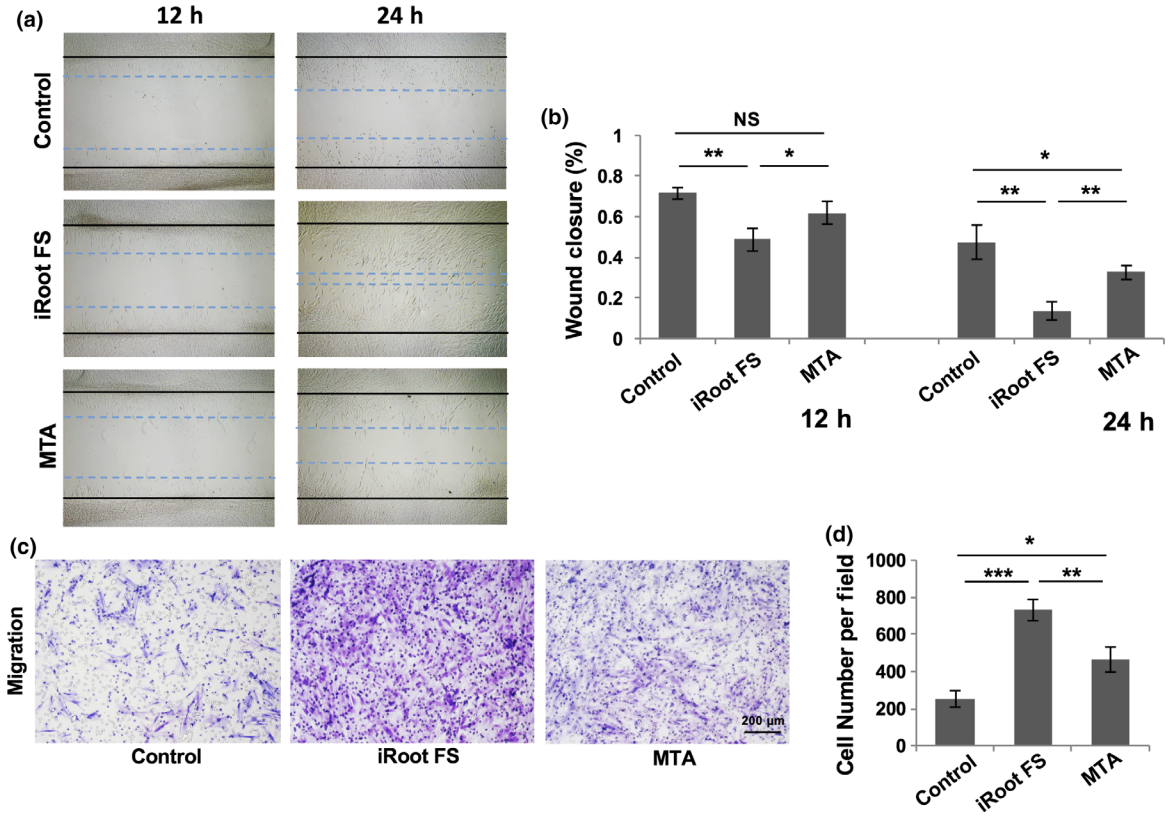


**Figure 3** iRoot FS improved the cell migration of hSCAP. (a,b) Wound healing assay showed that the wound closure percentage of hSCAP cultured in iRoot FS conditioned medium was significantly decreased when compared with the MTA group (**$P < 0.01$) and control group (**$P < 0.01$). (c,d) Transwell cell migration assay showed that the number of migrated cells in iRoot FS group was higher than that of the MTA group (**$P < 0.01$) and control group (***$P < 0.005$). NS = no significant difference, Error bars: means ± SD

have been identified as the most important cell source for pulp-dentine tissue and continued root development. Thus, the biological effect on SCAP is an important issue when evaluating root-end filling materials used during apexification. Cell viability and cell proliferation are the critical elements to assess the biocompatibility of dental materials. In this study, MTT was used first to evaluate cell viability and proliferation. hSCAP were exposed to iRoot FS conditioned medium for 4 days, and the proliferation rate of hSCAP on day 1, 2, 3, 4 was not changed significantly when compared with cells cultured in regular medium. These data demonstrated that iRoot FS is a safe and biocompatible material without cytotoxicity when assessed in laboratory studies. Some investigators reported that low concentrations of iRoot FS were superior at promoting cell proliferation, including dental pulp stem cells and osteoblasts compared to MTA and Biodentine (Lv *et al.* 2017, Sun *et al.* 2017). Appropriate release of calcium and silicon could activate mitochondrial matrix dehydrogenases and facilitate cell proliferation (Contreras & Satrústegui 2009, Shie *et al.* 2014). The effects of iRoot FS on cell viability and proliferation might depend on concentration, culture system and cell type.

Progenitor cell migration plays essential roles in calcified tissue generation in apexification and the continued development of roots. It has been reported that MTA was able to promote cell migration of MSCs (D'Antò *et al.* 2010). In this study, hSCAP in the MTA group had elevated migration, consistent with the previous study. Moreover, hSCAP cultured in iRoot FS conditioned medium had greater numbers of migrated cells and more rapid wound closure than that cultured in MTA conditioned medium. Cell migration was influenced by material intervention, including physical and chemical mechanisms.

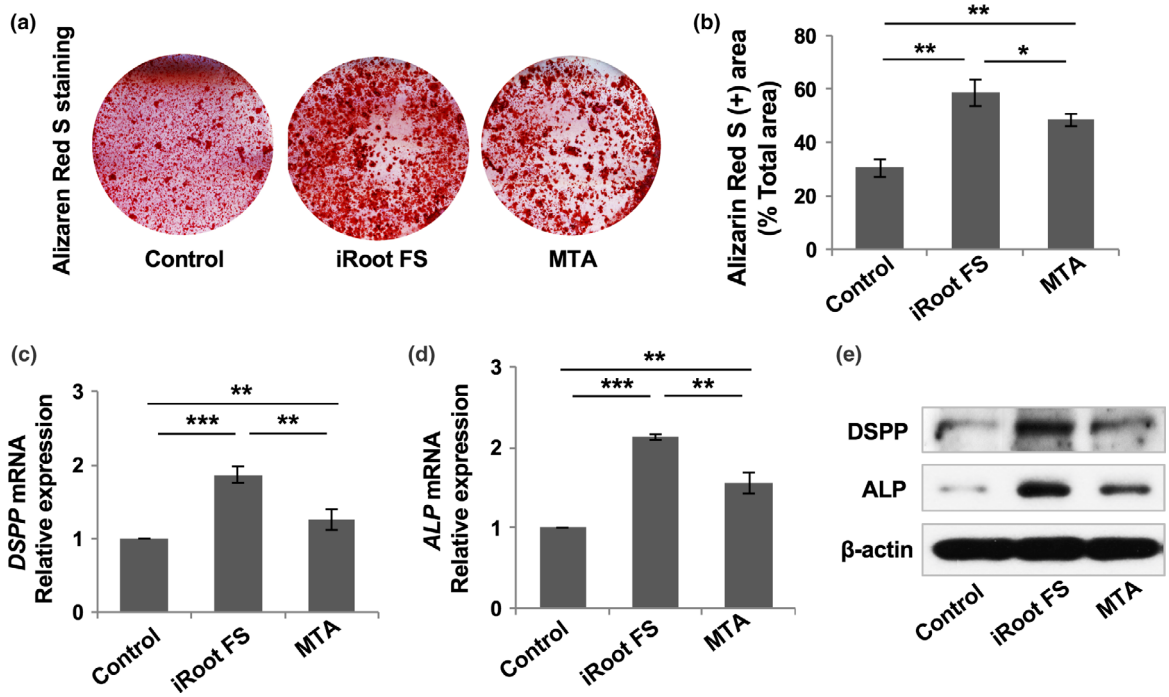


**Figure 4** iRoot FS improved the osteo/odontogenic differentiation of hSCAP. (a,b) Alizarin red S staining showed that iRoot FS increased the formation of mineralized nodules when compared with the MTA group (*$P < 0.05$) and control groups (**$P < 0.01$). (c,d) qPCR analysis showed that iRoot FS elevated the mRNA expression levels of *DSPP* and *ALP* of hSCAP when compared with the MTA group (**$P < 0.01$) and control group (***$P < 0.005$). (e) Western blot analysis showed that iRoot FS elevated the protein expression levels of DSPP and ALP of hSCAP when compared with the MTA and control group. Error bars: means ± SD

However, the detailed mechanism involved in the effects of iRoot FS on cell migration is unknown.

Both iRoot FS and MTA are calcium phosphate silicate cement (CPSC) (Lu & Zhou 2006). Several studies have indicated that CPSC materials have the potential to boost osteo/odontogenic differentiation by increasing the expression of osteo/odontogenic markers and calcium deposit (Peng *et al.* 2011, Sun *et al.* 2017, Luo *et al.* 2018). In the present study, the osteo/odontogenic differentiation of hSCAP exposed to both iRoot FS and MTA was enhanced markedly compared to the control group. Moreover, hSCAP cultured in iRoot FS conditioned medium had significantly greater expression levels of ALP and DSPP, and more formation of mineralized nodules than the MTA group. Those data demonstrated that iRoot FS was superior in facilitating osteo/odontogenic differentiation of hSCAP, consistent with previous studies (Peng *et al.* 2011, Sun *et al.* 2017, Luo *et al.* 2018). However, the detailed molecular mechanism of how iRoot FS promotes osteo/odontogenic differentiation is not reported. It was well known that Wnt is a key molecular in dentine and odontogenesis regeneration (Zhang *et al.* 2014). Classic Wnt/β-catenin signalling pathways operate at multiple stages of tooth morphogenesis, which is active in both dental epithelia and dentine, playing a key role in crown, root and periodontium formation, as well as controlling the shape of individual teeth (Liu *et al.* 2008). In this study, iRoot FS was able to elevate the expression of nuclear β-catenin to activate Wnt signalling pathway in hSCAP. Moreover, the Wnt/β-catenin inhibitor (XAV939) blocked the elevated osteo/odontogenic differentiation of hSCAP due to exposed iRoot FS through inhibiting the expression of nuclear β-catenin. These results indicated that iRoot FS enhanced the osteo/odontogenic differentiation of hSCAP *via* Wnt/β-catenin signalling.

Furthermore, iRoot FS is an alkaline material, and the pH value is similar to that of ProRoot MTA. The alkaline characteristic of iRoot FS may contribute to the antibacterial effects of the material in root canals. The ideal apical barrier should have favourable properties such as biocompatibility, insolubility, sealing

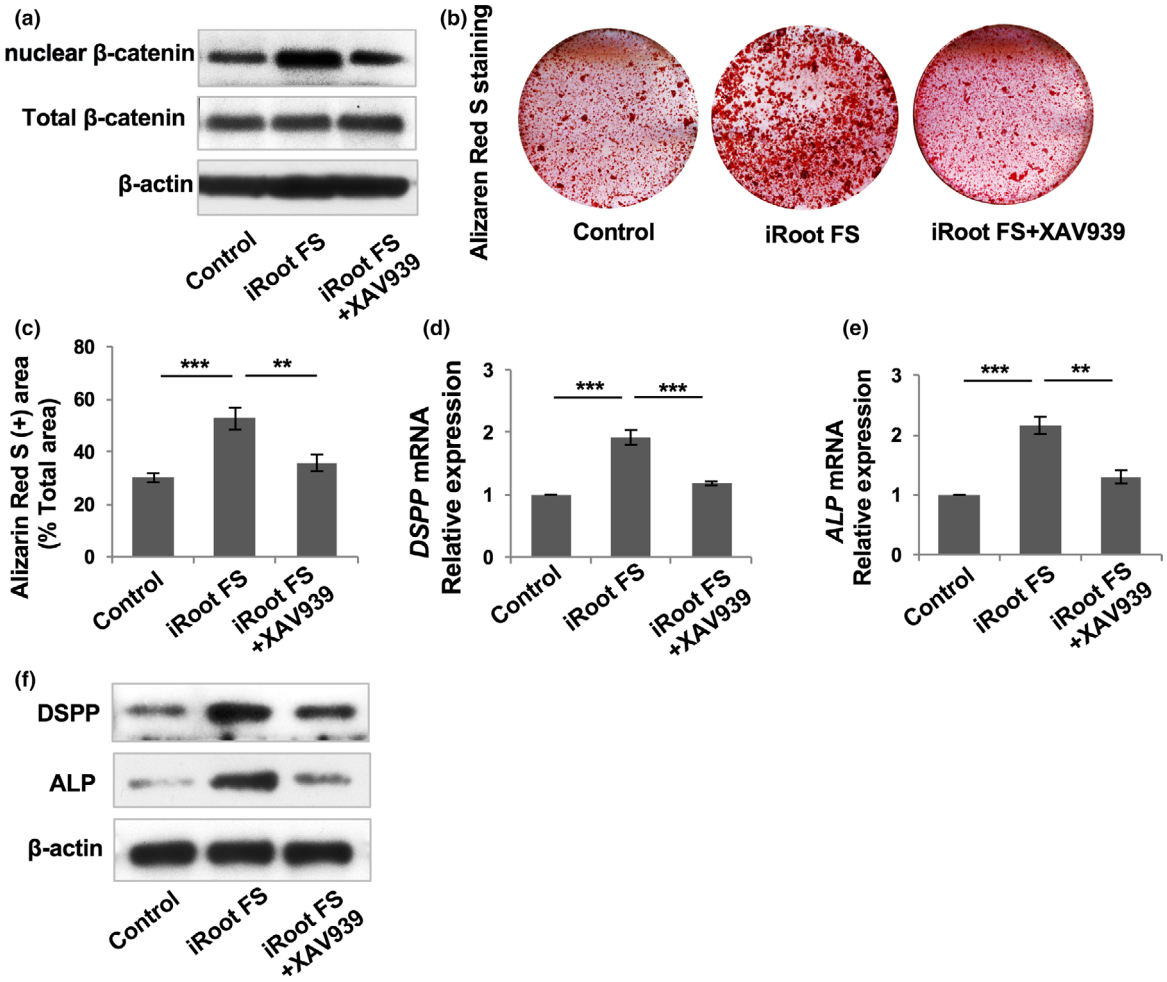


**Figure 5** iRoot FS enhanced the osteo/odontogenic differentiation of hSCAP *via* Wnt signalling. (a) Western blot analysis showed that the expression level of nuclear β-catenin was markedly increased in iRoot FS-treated hSCAP; while the Wnt inhibitor (XAV939) inhibited the expression levels of nuclear β-catenin. (b,c) Alizarin red S staining showed that mineralized nodule formation of hSCAP was elevated in iRoot FS-treated hSCAP (***$P < 0.005$), while XAV939 treatment blocked the elevated formation of mineralized nodules (**$P < 0.01$). (d,e) qPCR analysis showed that XAV939 treatment reduced the mRNA expression levels of *DSPP* (***$P < 0.005$) and *ALP* (**$P < 0.01$) of iRoot FS-treated hSCAP. (f) Western blot analysis showed that XAV939 treatment decreased the protein levels of DSPP and ALP of iRoot FS-treated hSCAP. Error bars: means ± SD

ability, radiopacity, antibacterial effects and the handing properties (Yan *et al.* 2010, Song *et al.* 2014). iRoot FS is a new-generation nanoparticle calcium phosphate silicate cement with superior mechanical characteristics and hydraulic properties (Zhou *et al.* 2013). iRoot FS contains tantalum pentoxide and zirconium oxide. Tantalum due to its inertness is used in sutures and membranes in orthopaedics, while zirconia is widely used to build prosthetic devices because of its excellent mechanical properties (Levine *et al.* 2006). Additionally, iRoot FS is able to completely solidify within 1 h at 37°C and has good cell adhesion and sealing ability (Jiang *et al.* 2014, Shi *et al.* 2015, Guo *et al.* 2016).

The treatment of immature permanent teeth with necrotic pulps aims to induce apical barrier formation and promote root development (Shabahang 2013). Regenerative endodontic treatment (RET) is another option for promoting continual root development in such teeth (Corbella *et al.* 2014). For a more

favourable prognosis of RET, a good coronal seal is critical, in addition to root canal disinfection and appropriate intracanal scaffolds. An ideal coronal sealing material should have appropriate characteristics, including excellent biocompatibility, no shrinkage and simplified handling without causing discolouration. Most importantly, it should promote the migration and osteo/odontogenic differentiation of stem cells homing to the root canal. The present data indicate that iRoot FS might have a potential use for coronal sealing in RET. So far, the biological evaluation of iRoot FS remains limited, and further animal experiments and clinical studies are needed to enrich knowledge on this nanoparticle bioceramic material and thus provide more evidence to support its clinical application.

## Conclusion

iRoot FS promoted cell migration of hSCAP and facilitate osteo/odontogenic differentiation of hSCAP *via* the Wnt/β-catenin signalling pathway without cytotoxicity. iRoot FS could be used as an apical barrier material in apexification or as a coronal sealing material in regenerative endodontic treatment.

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## Conflict of interest

The authors have stated explicitly that there are no conflicts of interest in connection with this article.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** iRoot FS treatment activated Wnt/β-catenin signaling pathway.