Bone marrow mesenchymal stem cells loaded β-tricalcium phosphate promote lumbar intervertebral fusion: a randomized controlled clinical trial

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Research Article

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Abstract

Background

As current stem cell tissue engineering always requires in vitro cell culture, it is subject to restrictions such as extra time cost, ethical problems, and contamination. A novel device named the bone mesenchymal stem cells screen-enrich-combine circulating system(SECCS) was designed to rapidly enrich stem cells and combine with β-tricalcium phosphate to immediately produce bioactive stem cells/β-tricalcium phosphate composites during surgery and implant them into the intervertebral space for intervertebral fusion.

Methods

37 patients who underwent transforaminal lumbar interbody fusion surgery were included in this study and randomly divided into two groups. One group underwent decompressed laminal bone graft for intervertebral fusion, and the other was given SECCS-prepared β-tricalcium phosphate particles. Cells adhered to β-tricalcium phosphate were eluted for stem cell identification, including scanning electron microscope observation, ALP staining, flow cytometry analysis, and induction for three-line differentiation. Bone marrow was collected before and after enrichment for efficient enrichment analysis. The fusion rate, decrease in intervertebral height, and functional improvement, including Japanese Orthopaedic Association and Oswestry Disability Index scores, were compared between the two groups preoperatively and at 3, 6 and 12 months postoperatively.

Results

The cells eluted from the β-tricalcium phosphate after screen-enrich-combine circulating system possessed characteristics of stem cell, including fiber-like morphology, positivity for CD44, CD73, CD90, and CD105, negativity for CD34, CD11b, CD19, CD45, and HLA-DR, and could been induced towards osteogenic, adipogenic, and chondrogenic differentiation. The screen-enrich-combine circulating system selectively enriched approximately 84.1% stem cells from the bone marrow in 6 minutes and could be simultaneously implanted into the intervertebral space during operation. Higher early fusion rate, similar intervertebral height decreases, and functional improvement tendencies were observed when stem cells/β-tricalcium phosphate particles were used as fusion scaffolds.

Conclusions

The screen-enrich-combine circulating system could quickly enrich stem cells from bone marrow, and the β-tricalcium phosphate particles prepared by the system showed higher early osteogenic efficiency than autologous laminal bone when used in intervertebral fusion, which provided a new bone scaffold substitute to autologous bone.

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1. Backgrouds

As common and frequently occurring diseases in orthopaedics, degenerative lumbar spine diseases (such as lumbar spinal stenosis, lumbar disc herniation, lumbar spondylolisthesis, etc.) often cause significant pain and even disability to patients. These complications not only negatively impact quality of life, but also impose a great economic burden on the family and society(1). Studies have shown that the high incidence of symptomatic lumbar degenerative diseases in Chinese adults (8.9%) is similar to that of chronic obstructive pulmonary disease (8.2%) and diabetes (9.7%)(2).

Increasing the interbody fusion rate is the most important goal of surgery for degenerative lumbar disease. Fusion failure may lead to a series of complications such as lumbar instability, pseudarthrosis formation, pedicle screw and rod breakage, and even cage retropulsion(3, 4). Although many factors are associated with poor fusion, the choice of bone graft material is undoubtedly crucial for determining its success. The most commonly used bone graft for intervertebral fusion is the decompressed lamina bone. Despite the disadvantage that it contains too much cortical and sclerotic bone, the decompressed laminal bone demonstrates a satisfactory fusion rate(5). However, in cases of infection, vertebral tumours, the laminal bone are not suitable for implantation. In addition, with the development and progress of minimally invasive technology, some new approaches have been developed, such as oblique lumbar interbody fusion and endoscopic translaminar lumbar interbody fusion (6). However, there is no autogenous bone graft for implantation for these surgical approach; thus, the iliac bone must be harvested. However, the bone harvest procedure results in complications such as pain at the bone harvest site, local hematoma, infection, nerve damage, and prolonged operation time(7). As a compromise, researchers are currently seeking a substitute scaffold for autologous bone grafts. An ideal bone graft material should have bone conduction, bone induction, and bone formation properties(8). In recent years, biomaterials and tissue engineering technologies have been rapidly developed. The use of new stem cell composite biomaterials to promote bone fusion has become a research hotspot and is gradually progressing towards clinical application. For example, Quarto et al reported a study in which mesenchymal stem cells (MSCs) were expanded in vitro and combined with porous hydroxyapatite particles, which successfully repaired three cases of large long bone defects. Of these cases, one had a defect of 7 cm in length; thus, this repair demonstrates the powerful effects of stem cell therapy(9).

Theoretically, stem cell-based tissue engineering technology has excellent application prospects for bone repair, and the results of laboratory and animal experiments are also promising. However, clinical translation is subject to many restrictions, such as in vitro culture-related contamination, ethical concerns, and additional time cost(10). To overcome these difficulties, our group developed a novel system called the bone marrow MSCs screen-enrich-combine circulating system (SECCS). The system can rapidly enrich bone marrow-derived MSCs and combine them with porous β-tricalcium phosphate (β-TCP), thereby preparing bioactive MSCs/β-TCP composites without the need for in vitro cell amplification. Since 2004, this technique has been successfully applied to treat bone nonunion and bone defects(11–13). However, we have not applied this technique in lumbar spine interbody fusion, and the role of the SECCS in promoting interbody fusion has not been reported. Therefore, we designed a randomized controlled

clinical study to use the SECCS to quickly and effectively combine MSCs with β-TCP for lumbar intervertebral fusion. We then observed the clinical outcome compared with traditional decompressed laminal bone graft (LBG). This research approach is expected to provide a new alternative method of bone grafting for lumbar interbody fusion.We present the following article in accordance with the CONSORT reporting checklist.

2. Methods

2.1 Selection criteria and baseline data

From June 2019 to December 2020, patients who were diagnosed with degenerative lumbar diseases and were ready for the transforaminal lumbar interbody fusion(TLIF) procedure were recruited into this study. The inclusion criteria for patients were as follows: (1) aged from 18–65 years, regardless of whether patients were male or female; (2) diagnosed with degenerative lumbar diseases; (3) did not participate in another clinical study within three months; and (4) voluntarily participated and provided signed informed consent. The exclusion criteria were as follows: (1) fracture; (2) tumour; (3) infection; (4) severe osteoporosis; (5) revision surgery; (6) mental illness; (7) metabolic or immune dysfunction; (8) drug abuse; (9) severe primary diseases of vital organs; (10) allergy; (11) pregnancy and breastfeeding; or (12) poor compliance. After the patients signed the informed consent form, they were randomly divided into two groups using a previously established randomized number table. One group using decompressed laminal bone graft (LBG) for intervertebral fusion and another group received MSCs/β-TCP composites prepared by the SECCS for intervertebral fusion. Eventually 37 patients were enrolled into the study, of which 19 received LBG treatment and 18 received SECCS treatment (Figure 1). The detailed data of patients included into the two groups were demonstrated at table 1.

2.2 MSCs/β-TCP composite preparation

After general anaesthesia, the patient was placed in a supine position, and a 16-gauge bevel medullo-puncture needle was used to puncture the anterior superior iliac spine. Approximately 80 mL bone marrow was collected before surgery (Figure 2A). The SECCS system mainly consists of the following parts: (1) a detachable columnar double-layer filter box, which used for loading the porous biomaterial and haemofiltration; (2) a sealed pipeline for fluid circulation; and (3) a peristaltic pump that exerts a force to drive the continuous circulation of the bone marrow (Figure 2B–2D). When the procedure was started, 3~6 g β-TCP particles (Bio-Lu, Shanghai, China) with mechanical strength > 2 Mpa, porosity > 40%, a diameter of 1–3.5 mm, and a mean pore size of 500 ± 200 μm were placed in the inner box. The inner and outer filter boxes were then screwed together. Approximately 60 mL bone marrow was injected into the pipeline, and the power pump was started to circulate the bone marrow in the pipeline and filter it through the porous β-TCP (Figure 2E and F). After 6 min (60 r/min,50HZ), the MSC-enriched β-TCP (MSCs/β-TCP composites) particles were manufactured and ready for implantation. In addition, 5 mL bone marrow was collected before and after enrichment for routine blood tests.

2.3 Surgical procedure

After general anaesthesia, the patient was placed in a prone position, and sterile drapes were placed on the surgical field after disinfection. TLIF was performed as previously reported(14). Specifically, for patients allocated to the LBG group, the bone graft filled in the intervertebral fusion cage was decompressed laminal bone, while MSC/β-TCP composites were used for fusion in patients in the SECCS group. In addition, bilateral pedicle screws and rods were used for fixation.

2.4 Enrichment efficiency evaluation

The bone marrow was collected before and after enrichment for MSC counting and maintained in Alpha Minimum Essential Medium (Sigma, USA) with 10% foetal bovine serum (FBS; HyClone). Then, the suspension was added to a 6-well plate for adherent cell culture in an incubator with 5% CO_2 at 37 °C. The medium was refreshed every two days, and 10–14 days later, the culture medium was replaced with osteogenic induction solution (complete medium + 50 μM sodium ascorbate [Sigma] + 10 mM glycerophosphate [Sigma, USA] + 100 nM Mdexamethasone [Sigma, USA]) and cultured for another 10–14 days. Next, the cell colonies were observed under a microscope and then stained with alkaline phosphatase (ALP; Beyotime, Shanghai, China). The number of ALP-positive colony-forming units (CFUs/ALP^+) with a diameter of more than 2 mm were counted. The average number of CFUs/ALP^+ colonies before and after enrichment represents the difference between the numbers pre- and post-SECCS for each patient. Enrichment efficiency was formulated as $(Pre_{CFUs/ALP+} - Post_{CFUs/ALP+})/Pre_{CFUs/ALP+} \times 100\%$. For erythrocyte, leukocyte, and haemoglobin counts, the bone marrow pre- and post-SECCS was detected using a blood cell detector.

2.5 Cell count and cell viability test

Erythrocytes were eliminated using erythrocyte lysate (SCIGE, Shanghai, China) according to the manufacturer's instructions. The retained cells were resuspended and 10 μL of the resuspended solution was mixed with trypan blue and added to a cell counting plate (Invitrogen, USA). Cell number and cell viability were detected using a cell counting machine (Beckman Coulter, Brea, CA, USA).

2.6 Flow cytometry

The MSC/β-TCP composite granules that remained after surgery were collected, and the MSCs were eluted by pancreatic enzymes. The cells were resuspended and cultured in complete medium for 14 days. The cells were collected and labelled with CD44, CD73, CD90, CD105, CD34, CD11b, CD19, CD45, and HLA-DR antibodies (BD Biosciences, USA) to identify MSC markers by flow cytometry (Becton, Dickinson and Company, USA).

2.7 Osteogenic, adipogenic, and chondrogenic differentiation

As per the abovementioned procedure, the cells adhering to the wall were obtained and passaged, and the first-generation MSCs were collected. For osteogenic differentiation, the cells were cultured in osteo-inductive medium (Stemcell, Canada) for 21 days. They were then fixed with 95% absolute ethanol for 30 min and stained with Alizarin Red (Solarbio, USA) for 30 min at 37 C. For adipogenic differentiation, cells were cultured in adipogenic induction medium (Stemcell) for two days. The medium was then changed to the adipogenic maintenance medium containing 10 μg/mL insulin and cells were incubated for one day. After 14 days, the cells were stained with Oil Red O (Sigma). For chondrogenic differentiation, aliquots of 250,000 cells were added to serum-free medium, resuspended in a 15 mL tube, and centrifuged at 600 × g for 5 min. Then, 0.5 mL chondrogenic medium (Stemcell) was added into the tube. After 48 h incubation at 37 °C with 5% CO_2, the cell pellets were flipped into the tube and cultured for 28 d. Then, the pellets were fixed with 4% paraformaldehyde (Servicebio, Shanghai, China) for 15 min, followed by Alcian blue staining (Solarbio).

2.8 Scanning electron microscope (SEM) observation

Some MSC/β-TCP composite granules that remained after surgery were collected and divided into two parts, which were cultured in complete medium for 2 h and 2 weeks, respectively. Particles were fixed in 2% glutaraldehyde (Servicebio) for 2 h and washed three times with 0.1 M PBS (pH 7.4) for 10 min each time. Then, 1% osmic acid (Best-Reagent, Shanghai, China) was added to fix the particles again for 1 h, and particles were post-rinsed in 0.1 mol/L phosphate buffer (pH 7.2) for 1 h. Ethanol was added for stepwise gradient dehydration. Isoamyl acetate (Sigma) was added and mixed with ethanol at a ratio of 1:1 for replacement. After 10 min, the replacement solution was discarded and pure isoamyl acetate was added to soak for 15 min. Finally, the critical point drying method was used to dry the samples. After sputter-coating with a layer of gold, the specimens were placed under an SEM for observation.

2.9 Patient follow-up

All patients were routinely followed up within one week, three months (± 7 days), six months (± 15 days) and 1 year after surgery. The interbody fusion rate was evaluated using X-ray and computed tomography (CT) scans. According to dynamic radiographs (lateral flexion and extension), with the angle between the vertebral bodies less than 5°, and continuous trabecular bone formation in the segment analysed by CT is recognized as fusion. Fusion failure is determined to have occurred when the cage is loosened and shifted, there is a translucent band > 2 mm on the cage surface, and the angle between the vertebral bodies is > 5° on the dynamic radiographs [26]. Intervertebral height collapse can also reflect the fusion quality; as such, we observed the change in intervertebral height, which was calculated as follows: (anterior disc height + posterior disc height)/2. Functional improvement was evaluated using the

Japanese Orthopaedic Association (JOA) and Oswestry Disability Index (ODI) scores. As the accuracy of pedicle screw insertion affects function improvement, we also evaluated this factor by CT scan after surgery. The screw position was classified into four grades, as follows: grade 0, no branch; grade 1, only the threads outside the pedicle are less than 2 mm; grade 2, core screw diameter outside the pedicle or breach 2–4 mm; and grade 3, complete screw outside the pedicle(15). Two senior orthopaedic surgeons and one radiologist assessed the images, and the unanimously judged result of two or three doctors was confirmed to be the approved result.

2.10 Statistical analysis

SPSS 22.0 statistical software (IBM Corp., Armonk, NY, USA) was used for data management and statistical analysis. The measurement data are expressed as`X ± S, and the count data are expressed as rate (%). The data were tested for normality by the Shapiro–Wilk test. Two sample t-test or the paired t-test were used to compare normally distributed variables. Chi-squared test were used to compare the proportion. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1 The SECCS can successfully enrich MSCs from bone marrow

To confirm that the cells adhered to β-TCP were indeed MSCs, the cells eluted from TCP particles were collected and cultured for stem cell identification. We observed cell morphology by scanning electron microscopy, detected the MSC markers by flow cytometry, and identified their three-line differentiation ability. In fact, after SECCS processing, the white TCP particles became red, indicating that cells from bone marrow adhered to TCP, especially red blood cells (Figure 3A,B). By scanning the TCP immediately after SECCS, we confirmed that MSCs and many erythrocytes were captured, and the cell had not spread completely at this time. Furthermore, erythrocytes were not observed after culturing for 10 days, while MSCs had spread completely and demonstrated fibroblast-like morphology (Figure 3C,D). In order to confirm that the MSCs maintained their proliferation capacity, we cultured the TCP particles for 21 days and stained for ALP. The particles were all ALP-positive, suggesting that the MSCs were successfully grown on TCP particles (Figure 3E). In addition, cells eluted from β-TCP particles were successfully induced for osteogenic, adipogenic, and chondrogenic differentiation (Figure 3F–3H). Flow cytometry showed that the cells were negative for Lin (including CD34, CD11b, CD19, CD45, and HLA-DR) and positive for CD44 (99.99%), CD73 (100%), CD90 (97.9%), and CD105 (91.3%)(Figure 3I–M). These results demonstrate that the SECCS can successfully enrich MSCs from the bone marrow.

3.2 The SECCS demonstrated selective enrichment for MSCs

To evaluate the enrichment efficiency of MSCs by the SECCS, the bone marrow was collected pre- and post- enrichment to count the cell number and stem cell CFUs formation. First, we observed that cell viability was not reduced immediately after enrichment (Figure 4A). MSCs number was represented by the number of CFUs formed. After SECCS filtration, the number of CFUs (ALP-positive cells) was significantly reduced after enrichment (Figure 4B,C). There were 179.1 ± 19.7 CFUs/mL formed before enrichment and 28.6 ± 8.5 CFUs/mL formed after enrichment, of which the enrichment efficiency was 84.1 ± 3.8% (Figure 4D). We also observed other cell components in bone marrow, and found that platelets also showed significant enrichment (22.2 ± 20.2%), while other components including erythrocytes, leukocytes, and haemoglobin were not significantly reduced after enrichment (Figure 4E–4I).

3.3 The SECCS possesses a similar clinical effect to autologous bone grafts

Follow-up was conducted for 25 lumbar segments in the LBG group and 26 segments in the SECCS group. To exclude the effect of the accuracy of pedicle screw insertion on intervertebral fusion, we evaluated 88 screws in the LBG group and 88 screws in the SECCS group. There were 78 screws of grade 0 and 10 screws of grade 1 in the LBG group and 79 screws of grade 0 and 9 screws of grade 1 in the SECCS group; no difference was found between the two groups. Next, the intervertebral height was continuously monitored. We found that the intervertebral height was recovered after TLIF compared to pre-operation, and the height continuously decreased with time; however, a similar downward trend was observed in both groups (Figure 5A). Furthermore, even though the two groups acquired same fusion rate at 12 months for 100% fusion, while at early stage, cases in SECCS group showed higher fusion rate than LBG group (Table 2). In addition, the JOA score represented surgical improvement rate, and the ODI score represented low back pain intensity, both showed a similar increasing or decreasing trend between the two groups, respectively (Figure 5B,C). A typical case of a patient who received SECCS treatment is shown in Figure 5D–5H, which indicates satisfactory intervertebral fusion after 3, 6, and 12 months of follow-up.

4. Discussion

As early as 1936, Mercer proposed that the ideal method of spinal fusion is intervertebral fusion, which could enhance spinal stability(16). In contrast to the general bone union of a fracture, spinal interbody fusion occurs between two cortical bones of the superior endplate and inferior endplate, which is more difficult than general bone union. Therefore, the selection of bone graft materials is an important factor in this procedure. An ideal bone graft material needs to have the characteristics of bone conduction, bone induction, and osteogenesis. Iliac bone is the gold standard for bone grafting, but it requires re-operation and causes significant complications. Currently, the most commonly used interbody bone graft material is decompressed laminal bone; however, the laminal bone is majorly composed of sclerotic cortical bone, which has poor early revascularisation and bone induction(17). Besides, in some situations, such as the OLIF approach that requires a larger volume of bone graft, the decompressed lamina bone may not meet

the demand regardless of quality or quantity, thus new bone graft need to be harvest and always from iliac crest.

In 1995, Crane et al proposed the concept of bone tissue engineering, which uses natural or synthetic materials as a scaffold for cell transplantation(18). These materials are then implanted into the bone defect, and the scaffold eventually repairs the bone defect. As calcium phosphate ceramics are biocompatible, safe, cost-effective, and easy to obtain, they are widely used as bone substitutes and are commonly used as bone repair materials in clinical practice. The β-TCP used in this study is a synthetic bioceramic with the chemical formula $Ca_3(PO4)_2$. Its chemical composition is similar to that of inorganic bone components and possesses high compressive strength and biocompatibility(19). The absorption of the scaffold itself and bone formation speed influence bone regeneration; as such, β-TCP also possesses a relatively balanced speed(20). It is also a good biodegradable ceramic material that can provide a large number of calcium and sulfate ions, as well as a scaffold structure for bone regeneration. The porous structure of β-TCP results in a larger surface area for intercepting bone marrow MSCs and capturing them during blood circulation. We confirmed that approximately 80% of bone marrow MSCs can enrich the β-TCP scaffold via SECCS filtration. As reported previously, the combination of β-TCP and MSCs has good osteogenic properties and can trigger fast and robust bone formation when used for other methods(21). The combination not only provides osteogenic cells, but also secretes cytokines and growth factors that promote bone formation.

One of the current difficulties of stem cell therapy is the acquisition of sufficient cells, as there is a low quantity of MSCs in the bone marrow and most of cell are other undesired cells, therefor the major goal is to select and enrich. The most common method to obtain these cells is to separate MSCs and amplify them in vitro. Although this method can acquire a large number of MSCs, however, its greatest disadvantage is also its in-vitro nature, which requires extra time, extremely strict culture conditions, and secondary implantation. Our SECCS system is designed to avoid such restrictions, as it requires no additional in vitro cell culture. Even though this method cannot increase the number of MSCs in the bone marrow, it can capture as many MSCs as possible. β-TCP not only captures MSCs, but also provides them with a growth environment, which has been confirmed by our in vitro culture of MSC/β-TCP particles. We also found that MSCs seem to have a selectively higher ratio of adherence onto β-TCP than erythrocytes, leukocytes, and haemoglobin, which occurs via an unknown mechanism. Owing to this characteristic, the SECCS can selectively capture the majority of MSCs in the bone marrow. Compared to direct MSC injection, the SECCS helps MSCs adhere to β-TCP particles, thereby reducing the loss of cells. This may be the reason why fewer cells are required for this method compared to for in vitro cell culture(22).

The principle of the SECCS is that the bone marrow blood circulates in the sealed pipeline, and we have confirmed that such a procedure does not affect cell viability. In our previous report, we found that five rounds in 10 minutes of circulation leads to saturated MSC enrichment(11). In this study, we further optimised the circulation speed and achieved a faster enrichment time by approximately 6 min circulation with a peristaltic pump at 60 rounds per minute, thereby allowing rapid formation of the MSC/β-TCP particles for implantation. The MSCs enrichment rate can reach over 80%, and the cells maintain

proliferation viability and the MSC-specific characteristic of osteogenic differentiation in vitro, thereby promoting interbody fusion. Unexpectedly, we observed that not only MSCs showed high adhesion to β-TCP; platelets also seemed to prefer to adhere to β-TCP, with an enrichment rate of approximately 22%. A recent study showed that platelet-rich plasma (PRP) can promote tissue repair, wound healing, and bone repair by the abundant cytokines released by platelets, including platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF), epithelial growth factor (EGF), and other growth factors(23, 24). It is unknown whether the platelets enriched from bone marrow promote fusion, as the enrichment rate was not as high as that observed for MSCs. However, we believe that fusion promotion can be amplified when MSCs are enriched accompanied with platelets.

It is currently common for stem cells to be combined with artificial bone for use in orthopaedic surgery; however, they are rarely used for spinal fusion. For example, in a study by Zheng, a mouse L4/L5 interbody fusion model was established, and bone marrow MSCs were seeded on a collagen sponge scaffold and cultured in osteoblast induction medium for three weeks to prepare a cell-scaffold complex that was implanted in the intervertebral space. After 12 weeks, bone marrow MSCs could induce osseous fusion in the lumbar vertebrae(25). Lu (26)used New Zealand rabbits with L3–L6 intervertebral discs removed, and bone marrow MSCs were cultured on porous tantalum implants and then used for intervertebral body fusion for comparison with autologous bone transplantation. The results showed that the radiographic fusion index scores at 12 months post-operatively were not significantly different between the autograft and tantalum groups. In another study, Li (27)removed the intervertebral disc of the rat tail vertebra and constructed a spinal intervertebral fusion model. In this study, porous scaffolds made of polycaprolactone and nano-hydroxyapatite were loaded with bone marrow MSCs that were treated with a sinusoidal electromagnetic field (EMF). The results revealed that the scaffold loaded with MSCs was successfully stimulated by EMF-accelerated intervertebral fusion.Although all of these studies eventually revealed a satisfactory interbody fusion ratio, they all required in vitro MSC culture and were performed only in animals. Our study is the first to use stem cells and biomaterials for spinal intervertebral fusion in humans, by screen and enrich over 80% MSCs from bone marrow and loaded them into ß-TCP, we achieved similar fusion rates to those of autologous bone grafts, Importantly, the ß-TCP loaded MSCs showed higher bone formation capacity at early stage of intervertebral fusion, indicated that stem cell loaded ß-TCP may superior to autologous bone. It is our hope that the bone graft prepared by the SECCS can provide a substitute for autologous bone graft in intervertebral fusion. However, it must be noted that this study was not only aimed at normal TLIF surgery. In fact, under real clinical conditions, the volume of the decompressed laminal bone is sufficient to fill the cage and can acquire good fusion. The purpose of this study was to demonstrate the good quality of the SECCS for interbody fusion; as such, we selected the most common surgical approach, TLIF, as the subject of this study. In clinical practice, the procedure may be more suitable for diseases such as vertebrae tumor, infection, or osteoporosis, wherein decompressed laminal bone cannot be used as a bone graft. The SECCS may also be useful when the anterior or lateral surgical approaches are selected, in which the laminal bone is not available and the iliac bone must be harvested.

In conclusion, this study demonstrates that the novel SECCS technique involving the rapid production of bioactive MSC/β-TCP scaffold is fast, convenient, safe, and effective for lumbar intervertebral fusion. As such, this method could avoid complications caused by iliac bone harvest. Because of its similar bone regeneration efficiency to autologous bone grafts, the SECCS technique has broad prospects for clinical application, especially as a replacement for autologous bone.

Abbreviations

β-TCP :β-tricalcium phosphate

SECCS: Screen-Enrich-Combine Circulating System

MSCs: mesenchymal stem cells

LBG: laminal bone graft

TLIF: transforaminal lumbar interbody fusion

JOA: Japanese Orthopaedic Association

ODI: Oswestry Disability Index

EMF: electromagnetic field

Declarations

Ethics approval and consent to participate: The trial was conducted in accordance with the Declaration of Helsinki (as revised in 2013). The study was approved by the Translational Medicine Ethics Committee of Shanghai Ninth People's Hospital, Shanghai JiaoTong University School of Medicine (NO. 2016-179-T123) and informed consent was taken from all individual participants.

Consent for publication: Not applicable

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests: The authors declare that they have no competing interests.

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Authors' contributions: Jie Zhao and Changqing Zhao designed the whole study. Baozhi Ding, Yaokai Gan were responsible for the study search. Xin Wang, XiaoJiang Sun, Haijun Tian, Kai Zhang and Xiaofei Cheng conducted data extraction. Dingbao Zhi and Xin Wang drafted the paper, Jie Zhao revised it. All authors read and approved the final manuscript.

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References

1. Martin BI, Mirza SK, Spina N, Spiker WR, Lawrence B, Brodke DS. Trends in Lumbar Fusion Procedure Rates and Associated Hospital Costs for Degenerative Spinal Diseases in the United States, 2004 to 2015. *Spine (Phila Pa 1976)* 2019;44:369–376.
2. Tian W, Lv Y, Liu Y, Xiao B, Han X. The high prevalence of symptomatic degenerative lumbar osteoarthritis in Chinese adults: a population-based study. *Spine (Phila Pa 1976)* 2014;39:1301–1310.
3. Kovač V. Failure of lumbar disc surgery: management by fusion or arthroplasty? *Int Orthop* 2019;43:981–986.
4. Li N, Dai M, Zhang B, He D, Wei Y, Duan F, Sun Y, Liu B, Mo F, Tian W. Risk factors for cage retropulsion after transforaminal lumbar interbody fusion in older patients. *Ann Transl Med* 2020;8:1660.
5. Han S, Park B, Lim J-W, Youm J-Y, Choi S-W, Kim DH, Ahn DK. Comparison of Fusion Rate between Demineralized Bone Matrix versus Autograft in Lumbar Fusion : Meta-Analysis. *J Korean Neurosurg Soc* 2020;63:673–680.
6. Momin AA, Steinmetz MP. Evolution of Minimally Invasive Lumbar Spine Surgery. *World Neurosurg* 2020;140:622–626.
7. Shin SR, Tornetta P. Donor Site Morbidity After Anterior Iliac Bone Graft Harvesting. *J Orthop Trauma* 2016;30:340–343.
8. Hasan A, Byambaa B, Morshed M, Cheikh MI, Shakoor RA, Mustafy T, Marei HE. Advances in osteobiologic materials for bone substitutes. *J Tissue Eng Regen Med* 2018;12:1448–1468.
9. Quarto R, Mastrogiacomo M, Cancedda R, Kutepov SM, Mukhachev V, Lavroukov A, Kon E, Marcacci M. Repair of large bone defects with the use of autologous bone marrow stromal cells. *N Engl J Med* 2001;344:385–386.
10. Perić Kačarević Ž, Rider P, Alkildani S, Retnasingh S, Pejakić M, Schnettler R, Gosau M, Smeets R, Jung O, Barbeck M. An introduction to bone tissue engineering. *Int J Artif Organs* 2020;43:69–86.
11. Wang X, Chu W, Zhuang Y, Shi D, Tao H, Jin C, Dai K, Zhao J, Gan Y. Bone Mesenchymal Stem Cell-Enriched β-Tricalcium Phosphate Scaffold Processed by the Screen-Enrich-Combine Circulating System Promotes Regeneration of Diaphyseal Bone Non-Union. *Cell Transplant* 2019;28:212–223.
12. Chu W, Gan Y, Zhuang Y, Wang X, Zhao J, Tang T, Dai K. Mesenchymal stem cells and porous β-tricalcium phosphate composites prepared through stem cell screen-enrich-combine(-biomaterials)

circulating system for the repair of critical size bone defects in goat tibia. *Stem Cell Res Ther* 2018;9:157.

13. Gan Y, Dai K, Zhang P, Tang T, Zhu Z, Lu J. The clinical use of enriched bone marrow stem cells combined with porous beta-tricalcium phosphate in posterior spinal fusion. *Biomaterials* 2008;29:3973–3982.
14. Ge DH, Stekas ND, Varlotta CG, Fischer CR, Petrizzo A, Protopsaltis TS, Passias PG, Errico TJ, Buckland AJ. Comparative Analysis of Two Transforaminal Lumbar Interbody Fusion Techniques: Open TLIF Versus Wiltse MIS TLIF. *Spine (Phila Pa 1976)* 2019;44:E555–E560.
15. Rajasekaran S, Bhushan M, Aiyer S, Kanna R, Shetty AP. Accuracy of pedicle screw insertion by AIRO® intraoperative CT in complex spinal deformity assessed by a new classification based on technical complexity of screw insertion. *Eur Spine J* 2018;27:2339–2347.
16. Mercer W. Spondylolisthesis: With a Description of a New Method of Operative Treatment and Notes of Ten Cases. *Edinb Med J* 1936;43:545–572.
17. Ott SM. Cortical or Trabecular Bone: What's the Difference? *Am J Nephrol* 2018;47:373–375.
18. Crane GM, Ishaug SL, Mikos AG. Bone tissue engineering. *Nat Med* 1995;1:1322–1324.
19. Tian Y, Lu T, He F, Xu Y, Shi H, Shi X, Zuo F, Wu S, Ye J. β-tricalcium phosphate composite ceramics with high compressive strength, enhanced osteogenesis and inhibited osteoclastic activities. *Colloids Surf B Biointerfaces* 2018;167:318–327.
20. Park JY, Park SH, Kim MG, Park S-H, Yoo TH, Kim MS. Biomimetic Scaffolds for Bone Tissue Engineering. *Adv Exp Med Biol* 2018;1064:109–121.
21. Gao P, Zhang H, Liu Y, Fan B, Li X, Xiao X, Lan P, Li M, Geng L, Liu D, Yuan Y, Lian Q, Lu J, Guo Z, Wang Z. Beta-tricalcium phosphate granules improve osteogenesis in vitro and establish innovative osteo-regenerators for bone tissue engineering in vivo. *Sci Rep* 2016;6:23367.
22. Gross J-B, Diligent J, Bensoussan D, Galois L, Stoltz J-F, Mainard D. Percutaneous autologous bone marrow injection for treatment of delayed and non-union of long bone: a retrospective study of 45 cases. *Biomed Mater Eng* 2015;25:187–197.
23. Farmani AR, Nekoofar MH, Ebrahimi Barough S, Azami M, Rezaei N, Najafipour S, Ai J. Application of Platelet Rich Fibrin in Tissue Engineering: Focus on Bone Regeneration. *Platelets* 2021;32:183–188.
24. Al-Maawi S, Dohle E, Lim J, Weigl P, Teoh SH, Sader R, Ghanaati S. Biologization of Pcl-Mesh Using Platelet Rich Fibrin (Prf) Enhances Its Regenerative Potential In Vitro. *Int J Mol Sci* 2021;22:2159.
25. Zheng W-D, Ming J-J, Chang W-L. The impact of collagen sponge composite bone marrow mesenchymal stem cells (BMSCs) in inducing interbody fusion. *Eur Rev Med Pharmacol Sci* 2018;22:5822–5827.
26. Lu M, Xu S, Lei Z-X, Lu D, Cao W, Huttula M, Hou C-H, Du S-H, Chen W, Dai S-W, Li H-M, Jin D-D. Application of a novel porous tantalum implant in rabbit anterior lumbar spine fusion model: in vitro and in vivo experiments. *Chin Med J (Engl)* 2019;132:51–62.

27. Li W, Huang C, Ma T, Wang J, Liu W, Yan J, Sheng G, Zhang R, Wu H, Liu C. Low-frequency electromagnetic fields combined with tissue engineering techniques accelerate intervertebral fusion. *Stem Cell Res Ther* 2021;12:143.

Tables

Table 1

Baseline data of the two groups

Baseline date					
		LBG	SECCS	c^2/t/Z	P
cases		19	18		
Gender				0.021	0.886
	Male(n)	8	8		
	Female(n)	11	10		
Age(years)		55.16± 8.07	54.50± 6.82	0.260	0.797
BMI		24.95± 2.76	25.00± 2.77	0.063	0.950
Fusion				0.218	0.640
	Single segment	13	10		
	Multi-segment	6	8		
Diseases				-.779	0.436
	Lumbar disc herniation	8	6		
	lumbar spinal stenosis	3	2		
	Spondylolisthesis	7	8		
	Degenerative lumbar scoliosis	0	2		
	Discogenic low back pain	1	0		

Table 2

Intervertebral fusion of the two groups

	3 months		6 months		12 months	
	LBG	SECCS	LBG	SECCS	LBG	SECCS
Successful	7	14	15	20	25	26
Failed	18	12	10	6	0	0
X2	3.515		1.695		1	
P-value	0.061		0.193		1.000	
Fusion rate	28.0%	53.8%	60.0%	76.9%	100%	100%

Figures

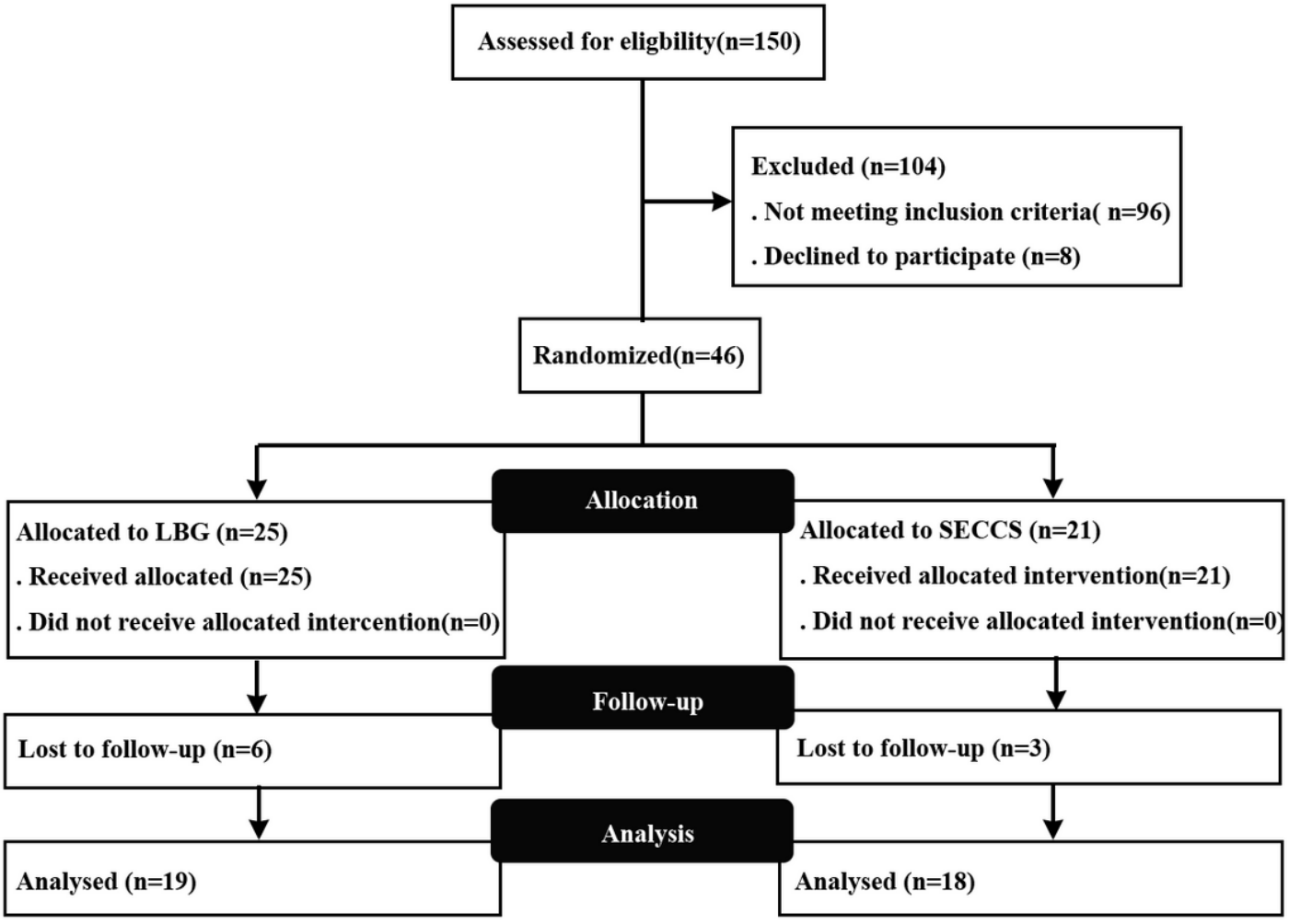

Figure 1

Trial Schema.

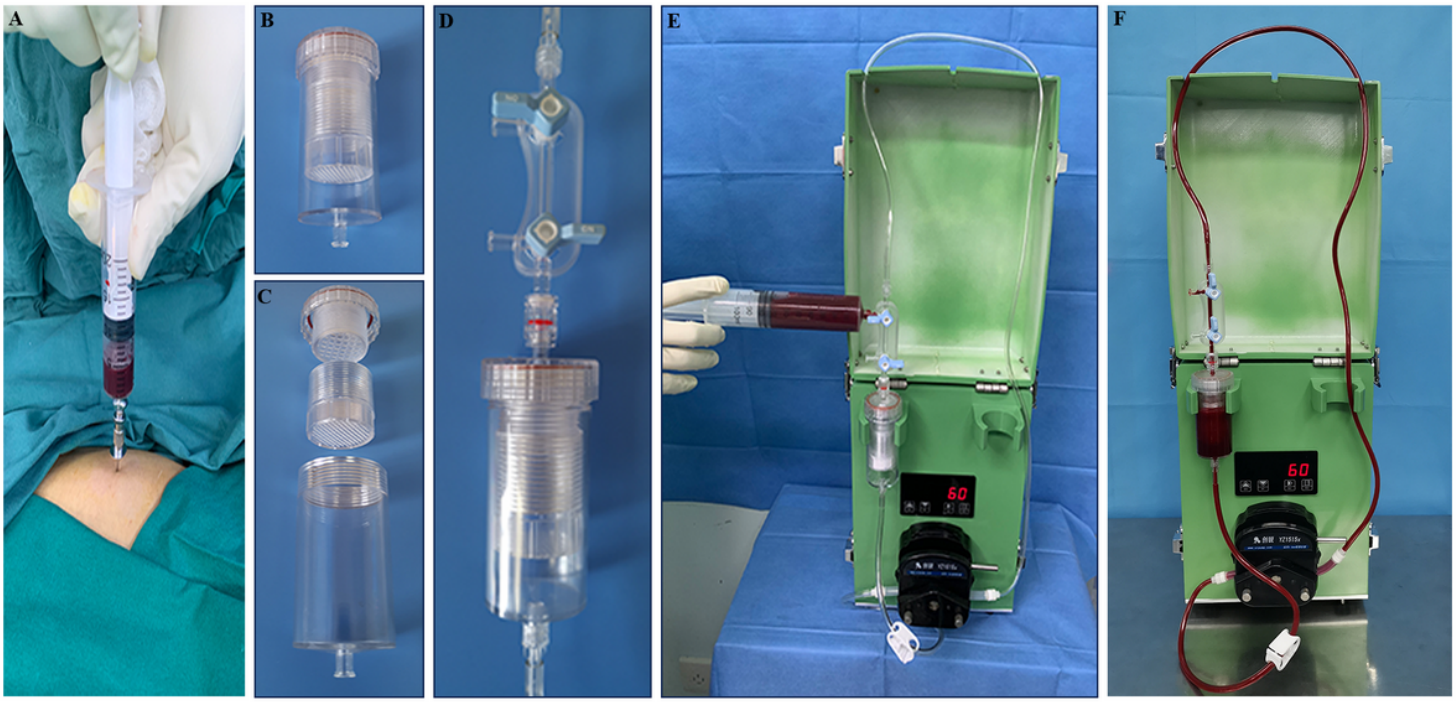

Figure 2

Major component parts of the SECCS. A. Bone marrow was aspired from iliac bone. B. The box was assembled for placement of TCP. C. The parts of the box were separated. D. The box was linked to three-way switch. E. Bone marrow was injected into the assembled circulation pipeline from the three-way switch. F. Bone marrow was circulated in the pipeline using a peristaltic pump.

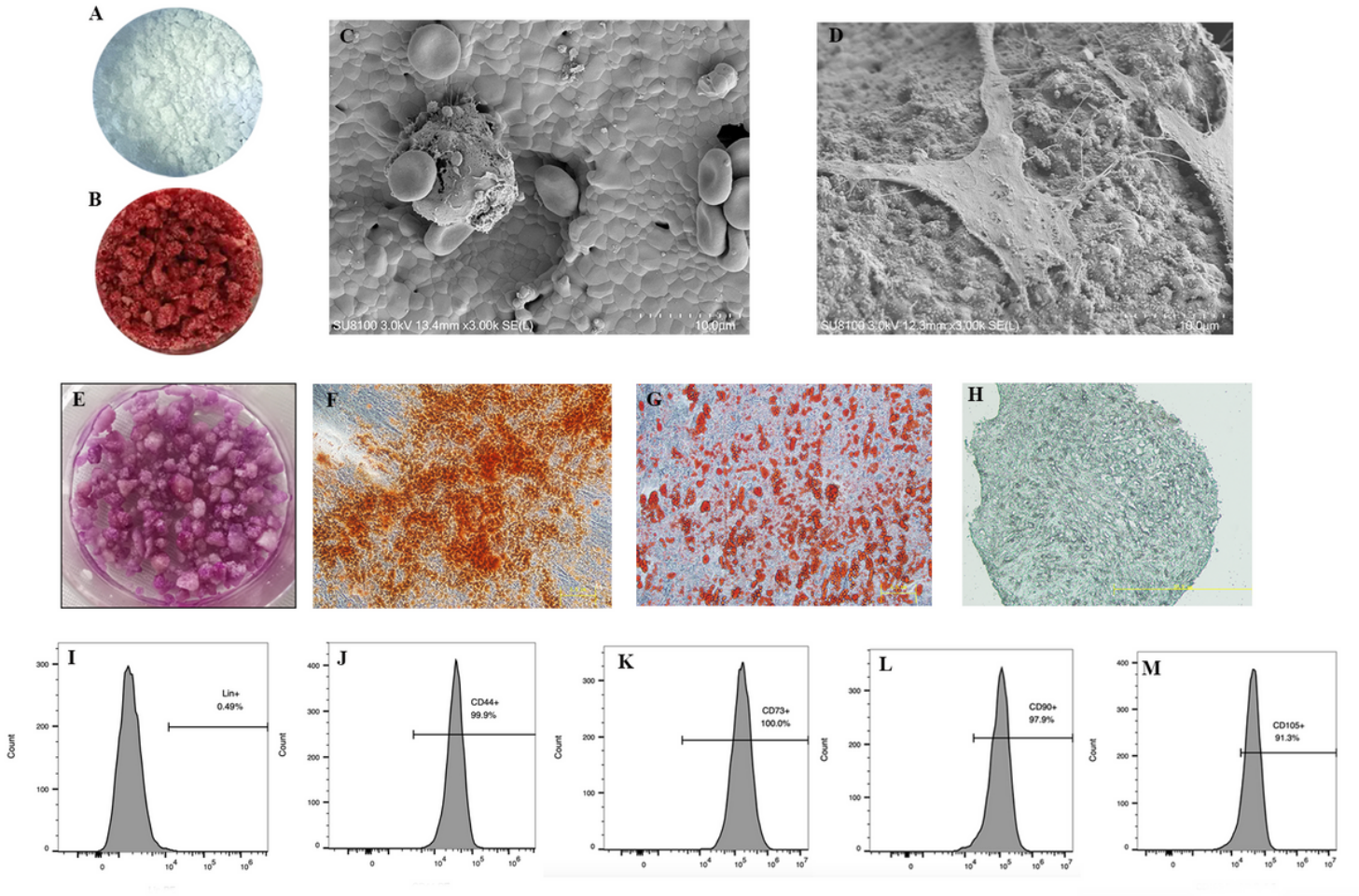

Figure 3

Identification of stem cells. A. β-TCP particles before preparation by the SECCS. B. β-TCP particles after preparation by the SECCS. C. Scanning electron microscope images of cells adhered to β-TCP immediately after preparation by the SECCS. D. Scanning electron microscope images of cells adhered to β-TCP after preparation by the SECCS and cultured for 10 days. E. ALP staining for β-TCP after preparation by the SECCS. F–H. Alizarin Red, Oil Red O, and Alcian blue staining of cells eluted from β-TCP. I–M: Cell surface markers of cells eluted from ß-TCP by flow cytometry.

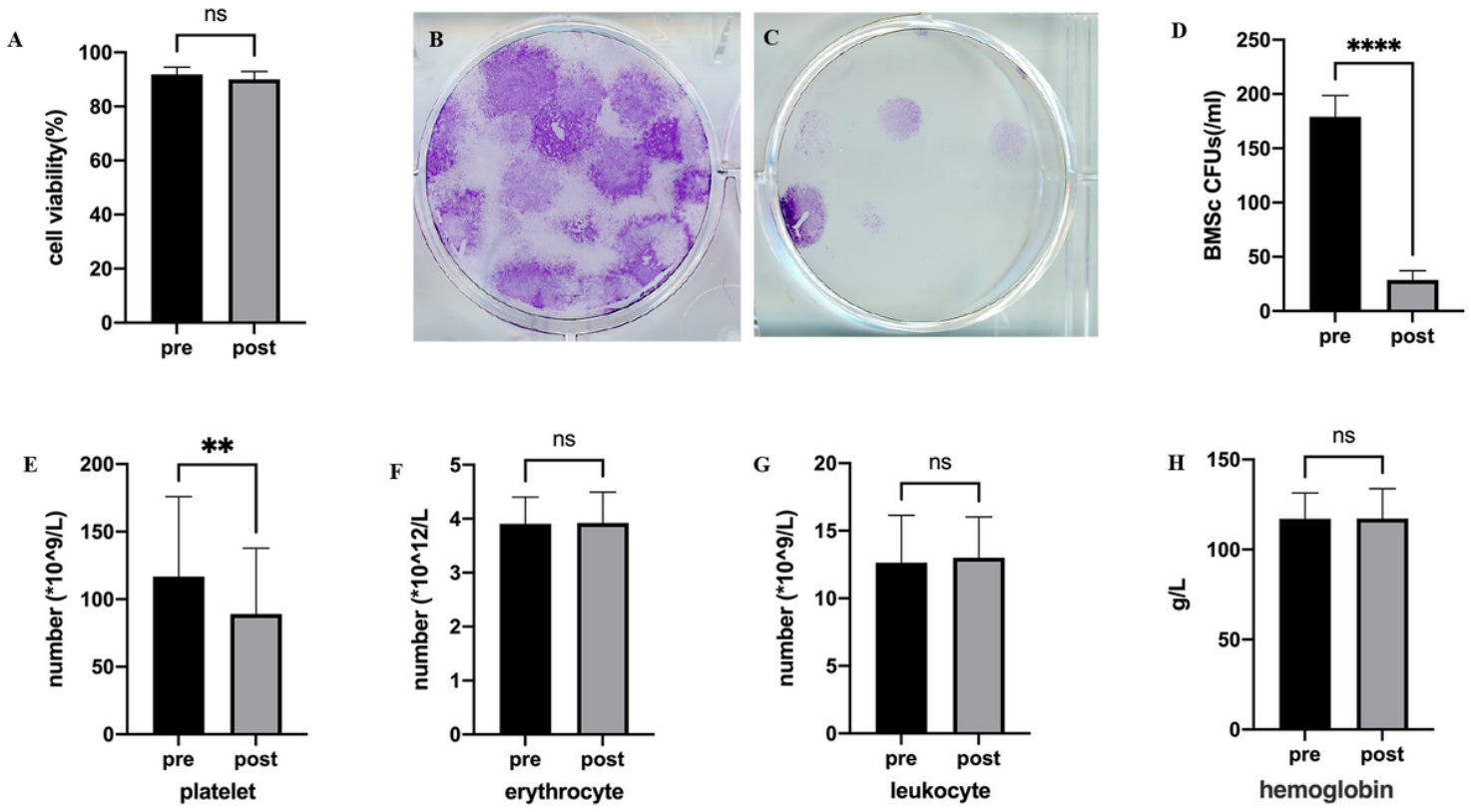

Figure 4

evaluation for the enrichment efficiency of SECCS. A: Cell viability pre- and post- enrichment. B, C: Colony-forming units from bone marrow pre- and post- enrichment. D–H: Changes in the different components of bone marrow pre- and post- enrichment.

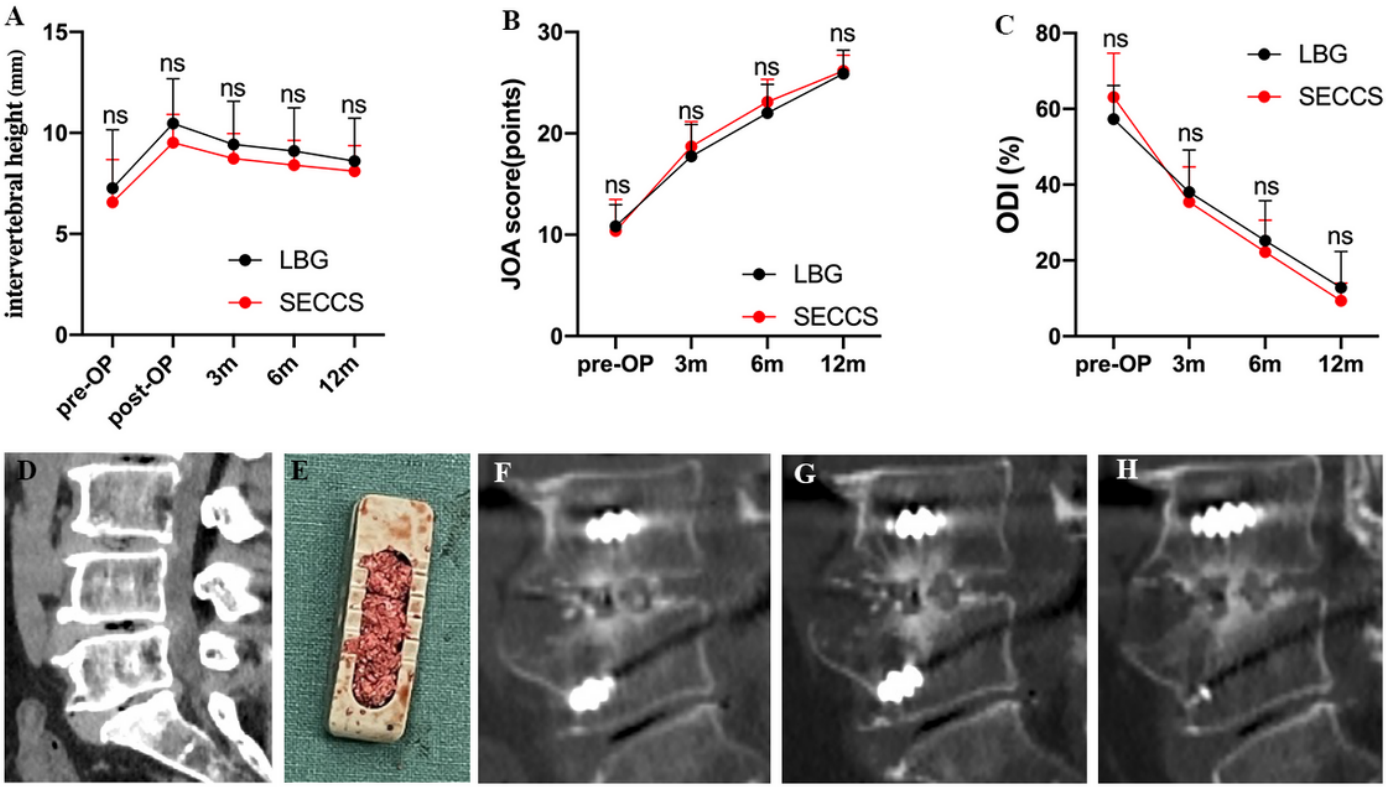

Figure 5

Clinical result for interbody fusion. A: Intervertebral height follow-up. B: JOA scores change by month of the two groups. C: ODI scores. D: CT scan of lumber spine before surgery. E: Intervertebral fusion cage filled with ß-TCP particles after preparation by the SECCS. F-G:Three-, six-, and twelve-month follow-up of intervertebral fusion by CT scan.