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Hertwig ’s Epithelial Root Sheath Cells Show Potential for Periodontal Complex Regeneration

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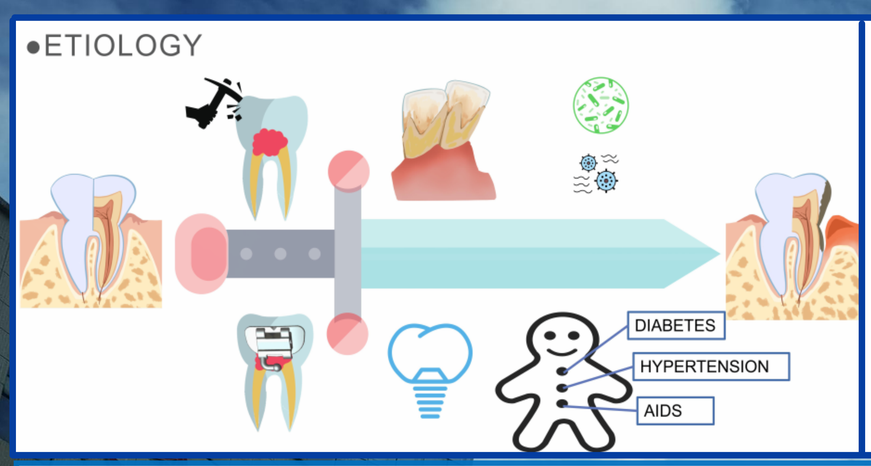
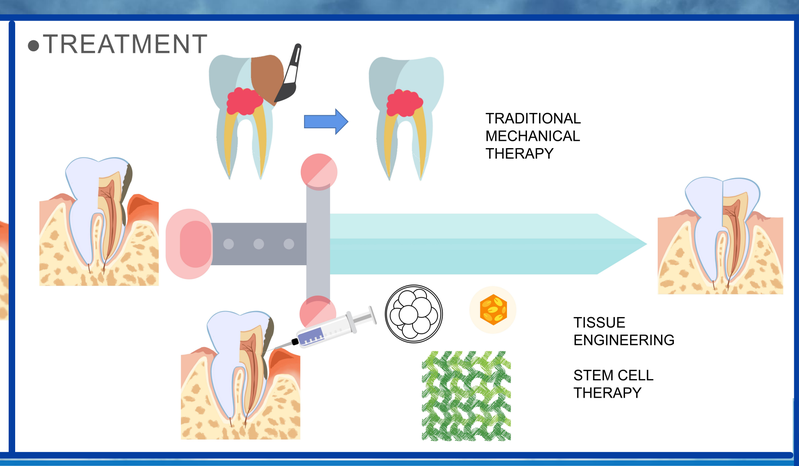
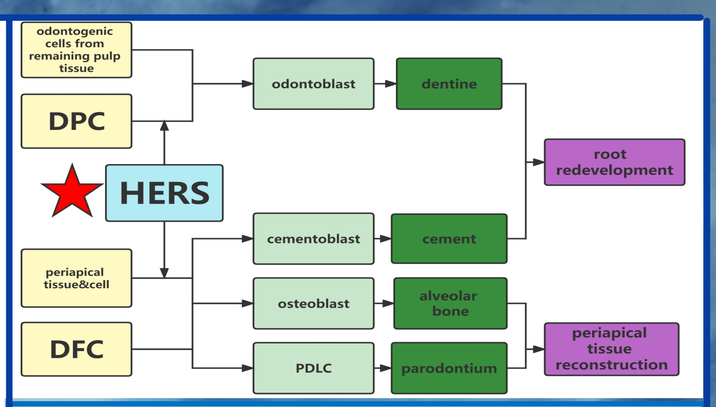






Pathological factors such as occlusal harshness, dental calculus with bacteria and systemic diseases might render normal periodontal tissue unhealthy, showing red and swollen gingiva, periodontal pocket, alveolar bone loss and even outer absorption of tooth root. Nowadays, researchers have paved their way to new therapeutic strategies of treating periodontal tissue loss, including the application of stem cells.

Hertwig ’s epithelial root sheath (HERS) cells, from the perspective of natural tooth root and periodontal development, are able to induce dental follicle cells (DFCs) to differentiate into cementoblast, osteoblast and fibroblast to form cement, alveolar bone and parodontium. Meanwhile, the very cells are capable of rendering themselves differentiating into cementoblast via epithelial-mesenchymal transition and conduct cement forming and ossification process.

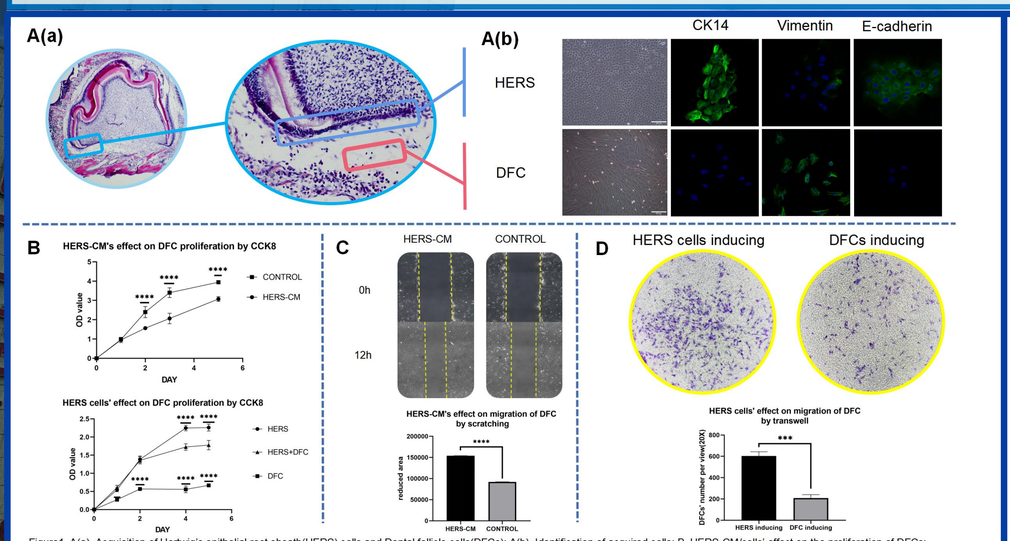


Figure1. A(a). Acquisition of Hertwig’s epithelial root sheath(HERS) cells and Dental follicle cells(DFCs); A(b). Identification of acquired cells; B. HERS-CM/cells’ effect on the proliferation of DFCs; C,D. HERS-CM/cells’ effect on the migration of DFCs.

Hers cell and dental follicle cell of 7d-old SD rat were successfully dislocated and cultured.Hers cell shows cobble stone like shape and positive for epithelial marker CK14 and E cadherin, while DFCs exhibit slender shape and positive for mesenchymal marker vimentin.
CCK8 assay shows that hers-conditioned medium inhibited the proliferation of dfc. Scratching test and transwell assay showed that hers conditioned medium/hers cells enhanced the migration of dfc.

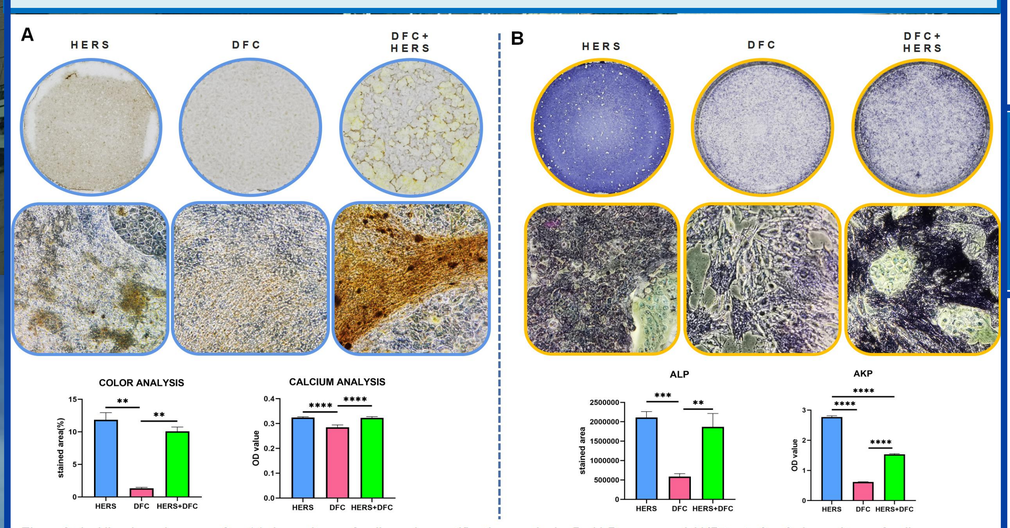


Figure2. A. Alizarin red assay after 14-day culture of cells and quantification analysis; B. ALP assay and AKP test after 3-day culture of cells

Alizarin red staining showed that in DFC alone group, calcification nodule can barely be seen; while in hers group, there are quite a lot stained nodules. Additionally, in hers+dfc group, the junction area between those two different type of cells contained a quite lot of mineralized nods. Color analysis and pyridine assay also support the fact that hers cell can promote the ossification.
ALP staining and AKP test results testified the mineralization ability of hers cells.

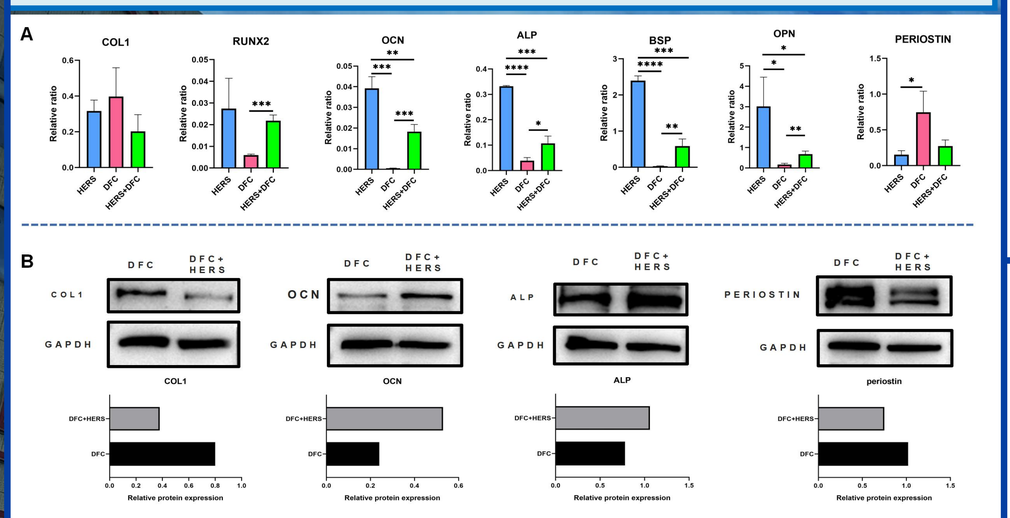
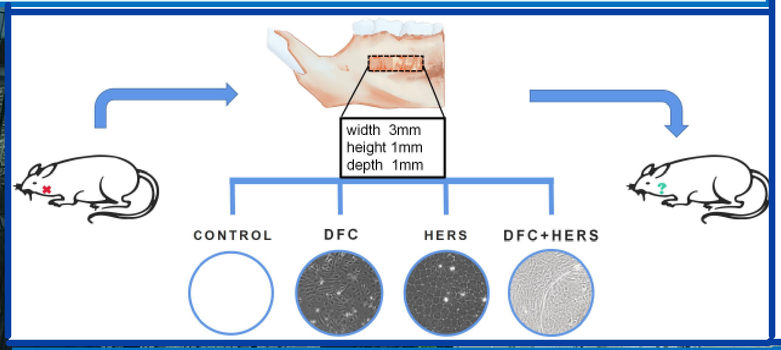


Figure3. A.real-time qPCR of osteogenic related gene expression after 3d culture of cells .B.Western blot of osteogenic related proteins after 3d culture of cells;

RT-qPCR results showed that HERS cells alone displayed the highest expression level of those osteogenic genes. HERS+DFC group also exhibited an enhanced trend of mineralization related genes ’ expression compared with DFC group.
WB also displayed higher level of ALP and OCN in hers+dfc group than dfc group.



A model of periodontal defect was also constructed to verify hers cell ability to induce periotissue repair.
A periodontal defect ranging width at 1mm\length at 3mm\depth at 1mm of the buccal side of manbibular first molar in 8W SD rat was fabricated. Then different groups of implants were placed in the defect.
Samples were collected for MICRO-CT scan&analysis and histology analysis.

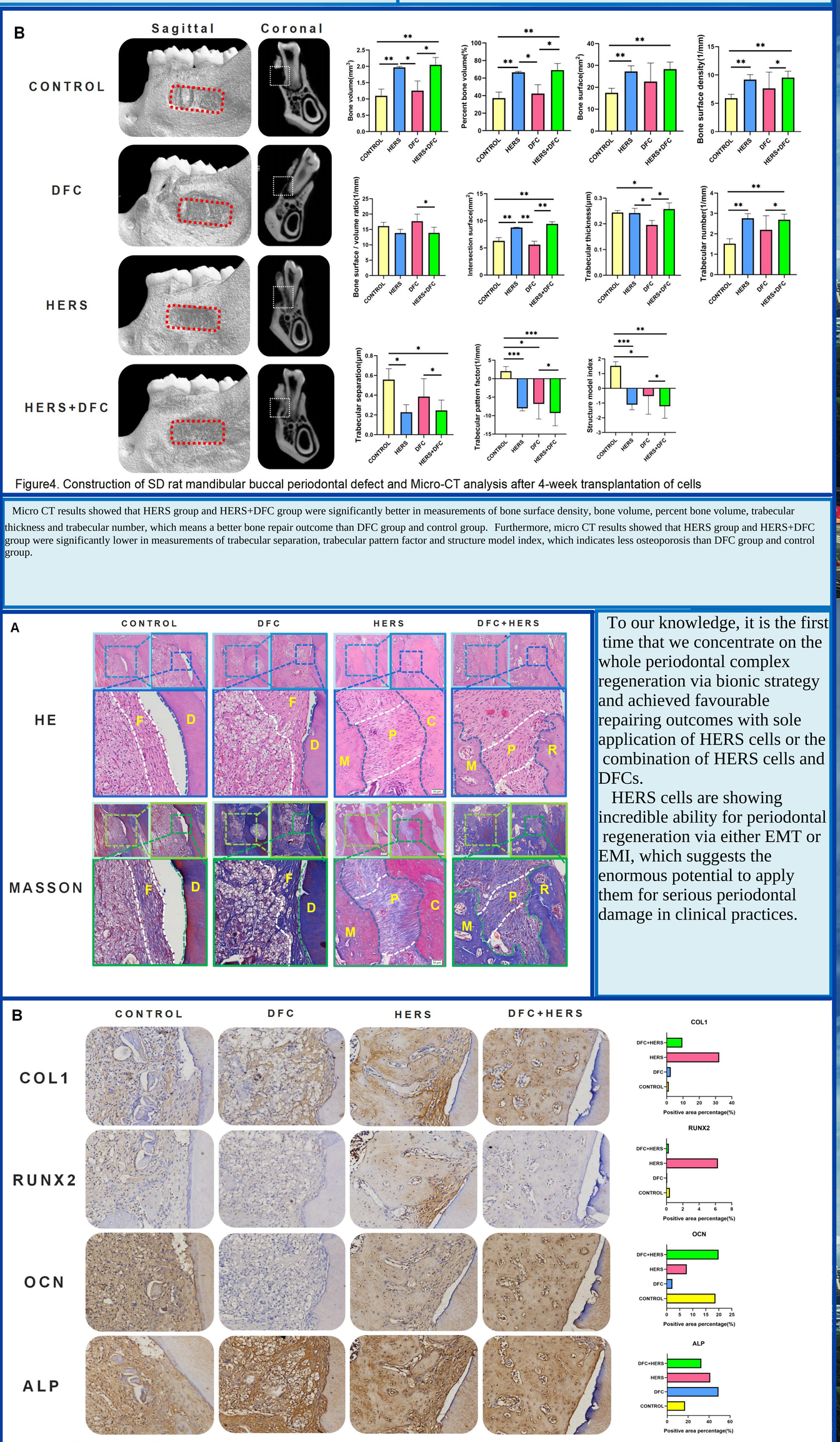


Figure4. Construction of SD rat mandibular buccal periodontal defect and Micro-CT analysis after 4-week transplantation of cells

Micro CT results showed that HERS group and HERS+DFC group were significantly better in measurements of bone surface density, bone volume, percent bone volume, trabecular thickness and trabecular number, which means a better bone repair outcome than DFC group and control group. Furthermore, micro CT results showed that HERS group and HERS+DFC group were significantly lower in measurements of trabecular separation, trabecular pattern factor and structure model index, which indicates less osteoporosis than DFC group and control group.

To our knowledge, it is the first time that we concentrate on the whole periodontal complex regeneration via bionic strategy and achieved favourable repairing outcomes with sole application of HERS cells or the combination of HERS cells and DFCs.
HERS cells are showing incredible ability for periodontal regeneration via either EMT or EMI, which suggests the enormous potential to apply them for serious periodontal damage in clinical practices.

Figure5. A. HE staining and MASSON staining; B. Immuohistochemical staining analysis

For histology analysis, HE staining, Masson staining and IHC were performed.
HE staining and Masson staining showed a lot more bone formation in the defected area of HERS group and HERS+DFC group than others. In addition, an complete structure of periodontal complex was observed in HERS group, showing cement attached to dentine, periodotium fibers arranging orderly at an certain angle anchoring from the inner side of cement to the outer side of alveolar bone and regenerative new bone.
IHC showed that the regenerated tissue of HERS group and HERS+DFC group exhibited more of the osteogenic-related proteins such as COL1, RUNX2, OCN and ALP.