Exploiting the power of decellularized ECM for periodontal tissue regeneration

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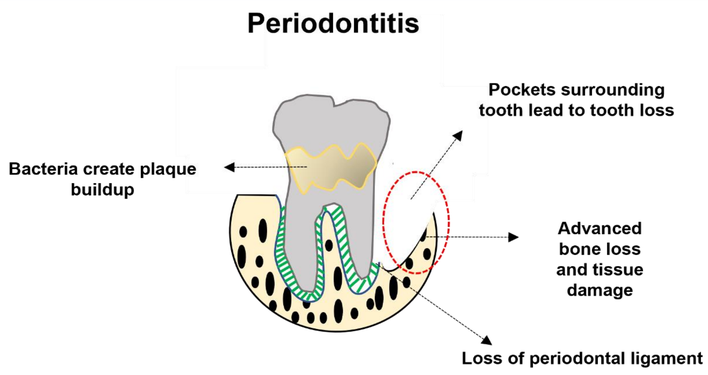
Motivation

Periodontitis is a prevalent disease worldwide that causes the destruction of periodontal tissues (**periodontal ligament**, **cementum** and **alveolar bone**) and ultimately can lead to tooth loss.

Most of the treatments, such as the use of membranes and bone grafts, **lack bioactive signals that accelerate the process of tissue regeneration**, leading to tooth loss.

In this work, we exploit alternative strategies to repair <u>all periodontal tissues</u> by using **decellularized extracellular matrix (ECM)** from **periodontal ligament stem cells (PDLSCs)**. We hypothesized that the PDLSC ECM incorporated into collagen sponges would enhance the **biofunctionality** of the scaffold and **periodontal regeneration**.

Background



Cell-derived ECM creates a **biomimetic microenvironment** that provides physical, chemical and mechanical cues for cells and supports **cell adhesion**, **proliferation**, **migration** and **differentiation**, **mimicking** the *in vivo* **cell niche**.

Results and Discussion

❖ Effect of decellularized ECM derived from PDLSCs

- **<u>Cell-derived ECM characterization</u>**

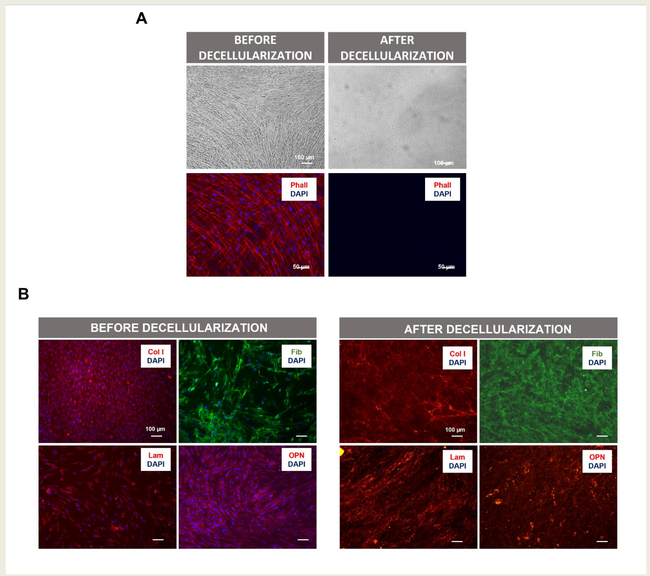


Figure 1: Characterization of decellularized ECM derived from PDLSCs. **(A)** Bright field and DAPI/Phalloidin images of PDLSCs before and after decellularization treatment. **(B)** Immunofluorescent staining images of ECM proteins collagen I (Col I), fibronectin (Fib), laminin (Lam) and osteopontin (OPN) before and after decellularization. DAPI was used to confirm the complete decellularization Scale bar, 100 µm.

- **<u>Cell proliferation</u>**

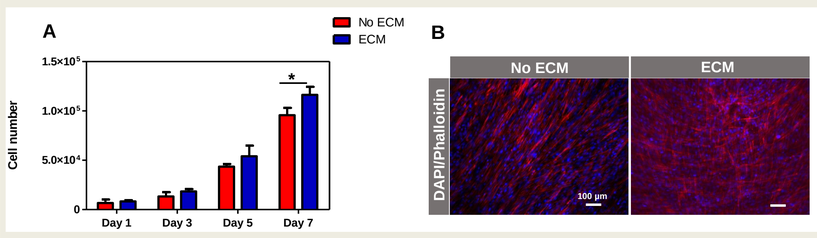


Figure 2: Effects of cell-derived ECM (PDLSC ECM) on PDLSC proliferation. **(A)** Cell numbers after 7 days of expansion. **(B)** PDLSC morphology after 7 days of expansion on PDLSC ECM (control-No ECM). Values are expressed as mean ± SD (n=3); *p < 0.05. Scale bar, 100 µm.

- **<u>Gene expression</u>**

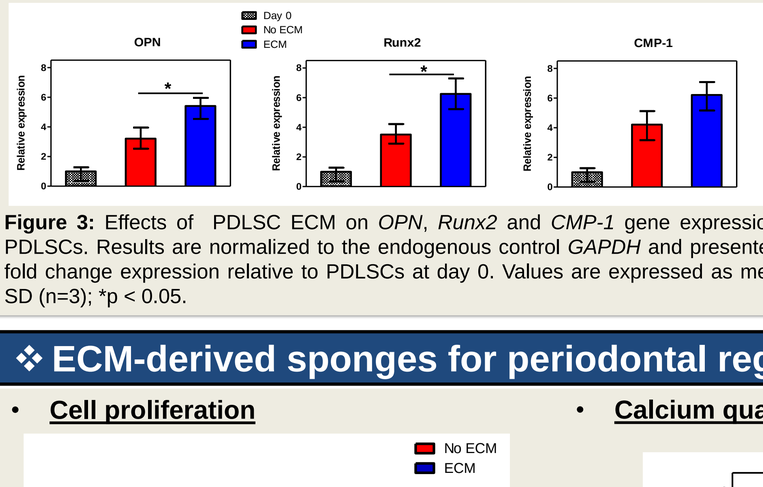


Figure 3: Effects of PDLSC ECM on *OPN*, *Runx2* and *CMP-1* gene expression by PDLSCs. Results are normalized to the endogenous control *GAPDH* and presented as fold change expression relative to PDLSCs at day 0. Values are expressed as mean ± SD (n=3); *p < 0.05.

- **<u>Mineralization</u>**

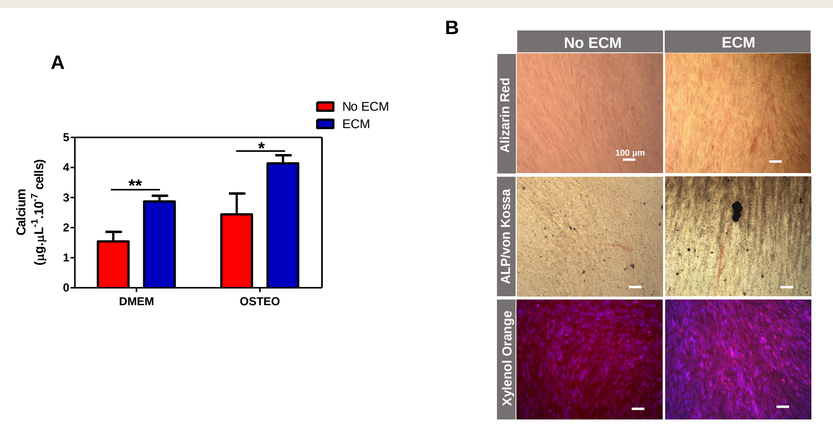


Figure 4: Osteogenic differentiation of PDLSCs cultured on decellularized ECM derived from PDLSCs. **(A)** Calcium deposition quantification of PDLSCs cultured on PDLSC ECM and without ECM after 21 days under osteogenic differentiation conditions (OSTEO) (control-DMEM). **(B)** Alizarin Red, ALP/von Kossa and Xylenol Orange stainings of PDLSCs differentiated on PDLSC ECM after 21 days. Alizarin Red staining confirmed the presence of calcium deposits (reddish areas). ALP/von Kossa staining demonstrated ALP activity of PDLSCs cultured on PDLSC ECM (reddish areas) and the presence of mineralized deposits (darker areas). Xylenol Orange fluorescent staining confirmed the presence of calcium deposits. DAPI was used to counterstain the cell nuclei in blue. Values are expressed as mean ± SD (n=3); *p < 0.05, **p < 0.01. Scale bar, 100 µm.

❖ ECM-derived sponges for periodontal regeneration

- **<u>Scaffold characterization</u>**

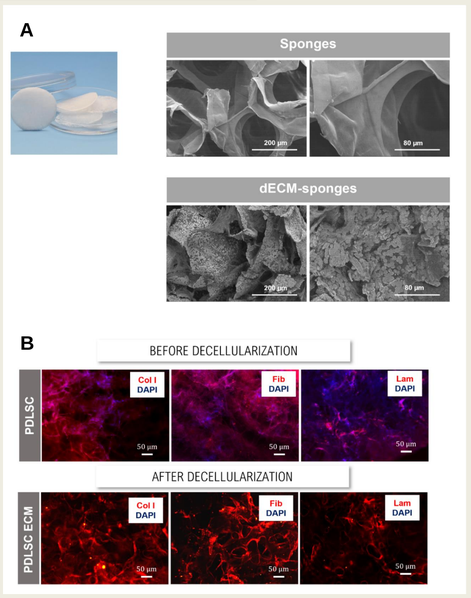


Figure 5: Decellularized ECM (dECM) – sponges for periodontal treatment. **(A)** SEM micrographs of collagen sponges enhanced with PDLSC ECM. **(B)** Immunofluorescent staining of collagen I, fibronectin and laminin produced by PDLSCs cultured on sponges before and after decellularization treatment. DAPI was used to confirm the complete decellularization. Scale bar, 50 µm.

- **<u>Cell proliferation</u>**

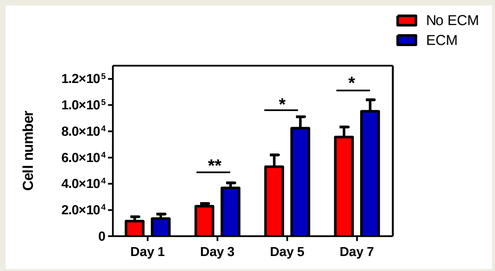


Figure 6: Effects of ECM sponges on PDLSC proliferation. Values are expressed as mean ± SD (n=3); *p < 0.05, **p < 0.01.

- **<u>Calcium quantification</u>**

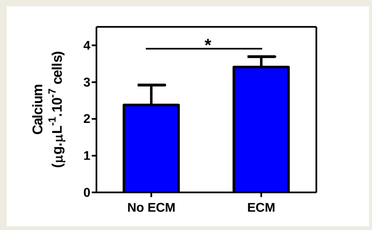


Figure 7: Calcium deposition quantification of PDLSCs cultured on ECM sponges after 21 days under osteogenic differentiation conditions. Values are expressed as mean ± SD (n=3); *p < 0.05.

- **<u>Osteogenic/Periodontal stainings</u>**

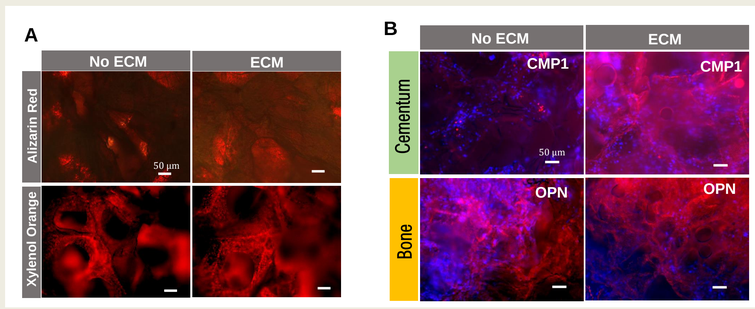


Figure 8: Osteogenic/Periodontal differentiation of PDLSCs cultured on ECM sponges. **(A)** Alizarin Red and Xylenol Orange stainings confirmed the presence of calcium deposits (reddish areas). **(B)** Immunofluorescent staining of cementum and bone ECM proteins (CMP-1 and OPN) confirmed the production of important periodontal ECM proteins by PDLSCs cultured on ECM sponges. DAPI was used to counterstain the cell nuclei in blue. Scale bar, 50 µm.

- **<u>Gene expression</u>**

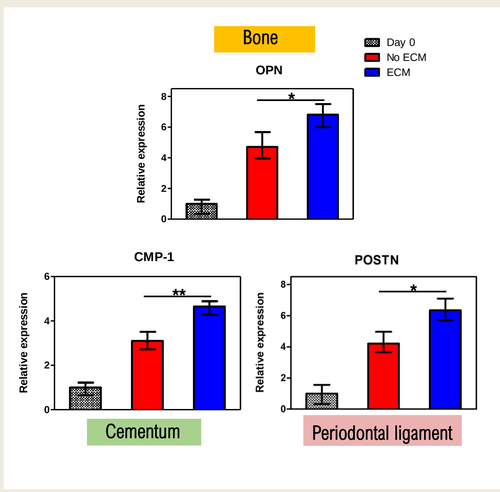
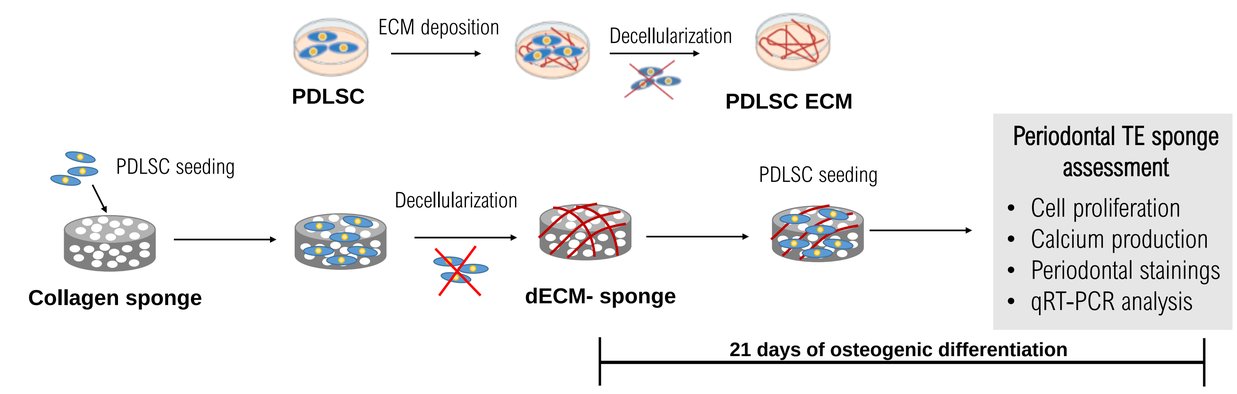


Figure 9: Effects of ECM sponges on *OPN*, *CMP-1* and *POSTN* gene expression by PDLSCs. PDLSCs cultured on ECM sponges upregulated the gene expression levels of bone (OPN)-, cementum (CMP-1)- and periodontal ligament (POSTN)-related genes. Results are normalized to the endogenous control GAPDH and presented as fold change expression relative to PDLSCs at day 0. Values are expressed as mean ± SD (n=3); *p < 0.05,**p < 0.01.

Materials and Methods

❖ PDLSC ECM production and fabrication of dECM-sponges



Conclusions

dECM-sponges have the potential to be used as **novel "off-the-shelf" biomaterials**, providing a **biomimetic microenvironment** that may contribute to **improve health care** of patients suffering with periodontal diseases.

PDLSC ECM → ✓ **Improved PDLSC proliferation** ✓ **Enhanced periodontal performance** → **Better mimicry of the *in vivo* periodontal ECM composition and structure**

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