Enhanced mechano-sensing of stem cells in dynamic hydrogels promotes the osteogenic differentiation

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Abstract

The biomaterials-assisted delivery of stem cells gives great advantages to the regeneration of dental tissues. Although the traditional system of static networks has been widely used, another alternative has emerged: structurally dynamic 3D hydrogels. This type of hydrogel responds to artificial activators and biological signals with spatial precision allowing them to better mimic the physicochemical properties of the cellular microenvironment. Herein, the synthesis of gelatin and acryloyl cyclodextrin hybrid hydrogels based on ”host – guest” interactions was achieved and the obtained hydrogels were used for the culture of human gingival fibroblasts (hGnF) in growth medium (GM) and osteogenic medium (OM) to study the osteogenic differentiation by examination of gene expression levels via PCR and IF of ALP, OCN, Col1, RunX2, and MAPK. Our results demonstrated that the cells cultured inside the dynamic hydrogels showed a significant differentiation inside the osteogenic medium.

Methods

First, 8 mg of gelatin and 10 mg of Ac-CD (acryloyl cyclodextrin) were dissolved in 50µl of PBS. Then, the hGnF cells were centrifuged to get the cell pellet in a 1.7 ml tube. Next, the precursor solution was mixed with the centrifuged cells, and the cells were suspended well by pipetting. Afterward, the mixed solution was transferred into a mold with a diameter of 5 mm. Finally, the mixed solution in the mold was exposed to ultraviolet (UV) light at an intensity of 10 mW/cm2 for 10 min. The obtained hydrogels were washed with PBS trice. Then, the hydrogels were cultured in the growth medium or the osteogenic medium, respectively. Next, we performed a check on the osteogenic gene expression by PCR and immunofluorescence (IF) staining with a focus on the markers: ALP, OCN, Col1, RunX2, and MAPK normalized to the expression level of GAPDH.

For the IF staining, we fixed the cells by incubation in paraformaldehyde for 15 min, followed by the permeabilization with Triton-X100 for 15 min. To block the unspecific binding of the antibodies, we further incubated the cells with bovine serum albumin for 2 h. Then, the cells were stained by the phalloidin, ALP or OCN antibodies, and 4′,6-diamidino-2-phenylindole (DAPI). The total mRNA was extracted by the TriZol method according to the manufacturer’s instructions. The obtained mRNA was re-

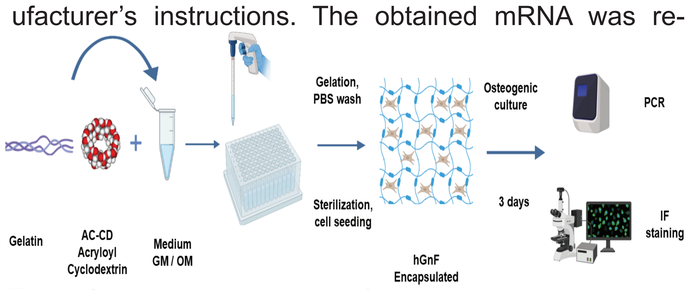

Fig. 1 Schematic illustration of dynamic preparation process.

Results

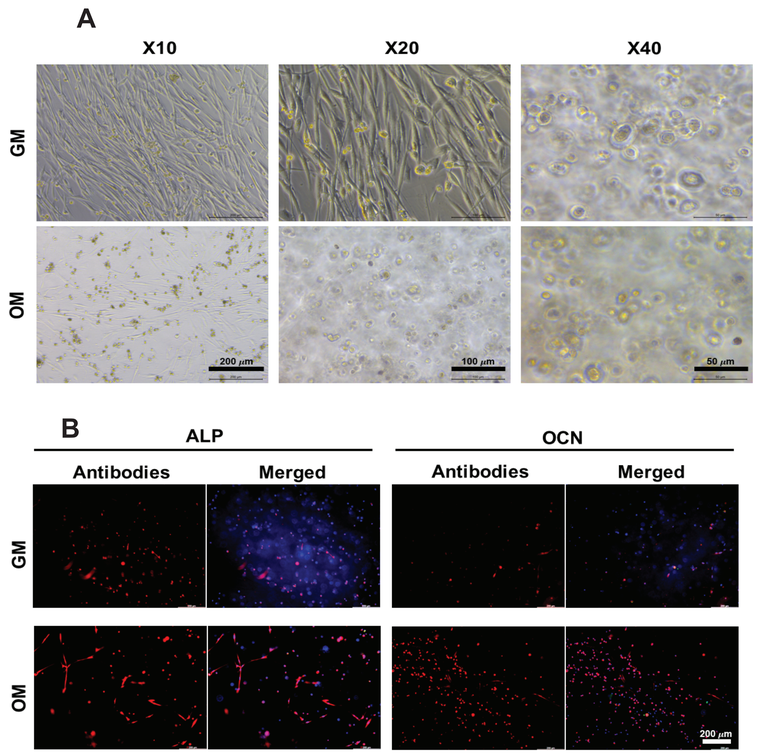

Fig. 2A hGnF fibroblasts morphology on GM and OM.
Fig. 2B Immunofluorescence staining results for ALP and OCN in GM and OM.

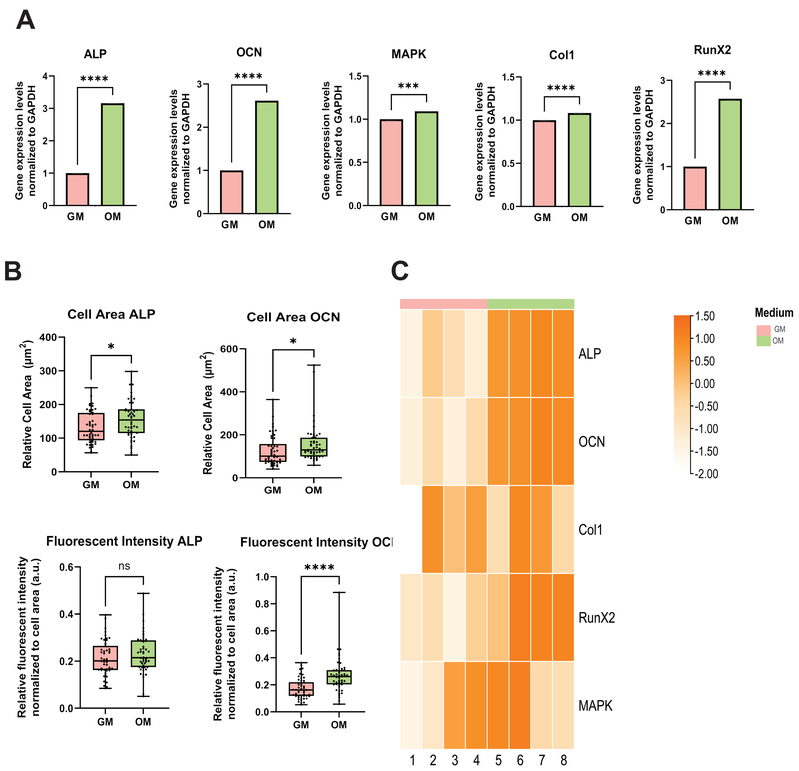

Fig. 3A Genes expression levels normalized to the expression of GAPDH.
Fig 3B Cell area and fluorescent intensity dependent on medium.
Fig 3C Heatmap of genes expression levels medium dependent.

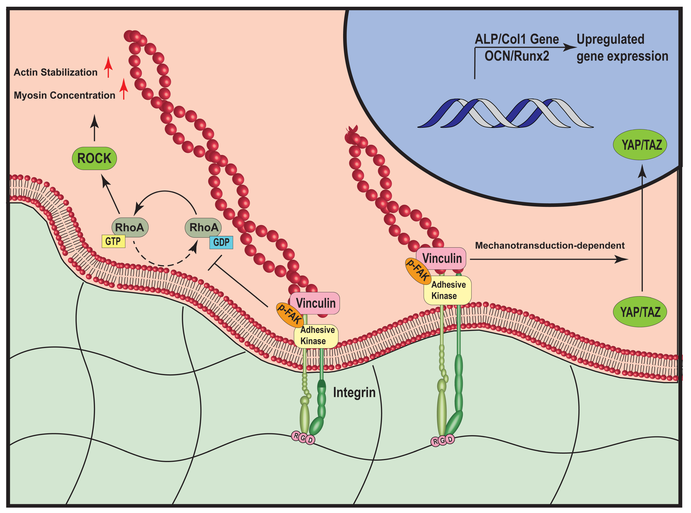

Fig. 4 The signal pathways involved in the enhanced cell adhesion and osteogenic differentiation.

Conclusions

It has been reported that the dynamic hydrogels would give a better supportive microenvironment for the osteogenic differentiation of stem cells. We cultured the stem cells in the dynamic hydrogels with OM or GM to investigate this effect. Interestingly, we found that our hydrogels can significantly promote cell adhesion and osteogenic differentiation as evidenced by the enlarged cell area and the expression of osteogenic markers: ALP, OCN, MAPK, Col1, and RunX2. Based on our findings, we speculated that the enhanced cell adhesion and osteogenic differentiation resulted from the enhanced matrix remodeling in the dynamic hydrogels, which is an inherent characteristic of the dynamic and reversible “host –guest” interactions. In the future, we reveal further deep into the detailed biomechanism by using more advanced molecular and cell biology techniques.

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