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Electronic Supplementary Information (ESI)

Preparation and characterization of keratin/chitosan UV-crosslinked composite film

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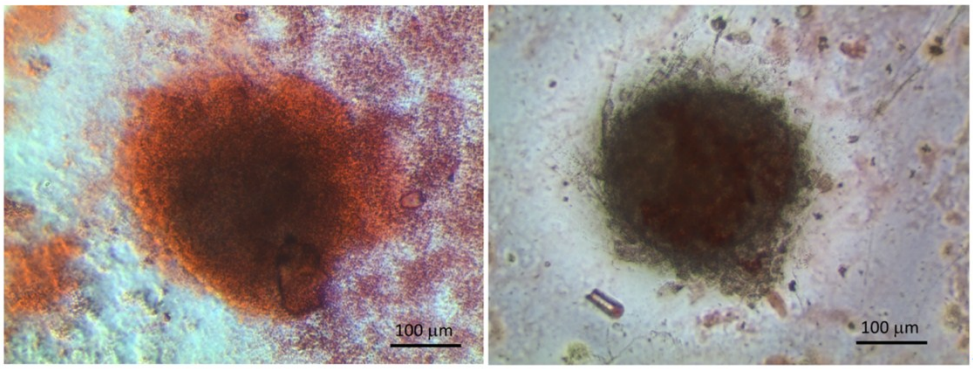
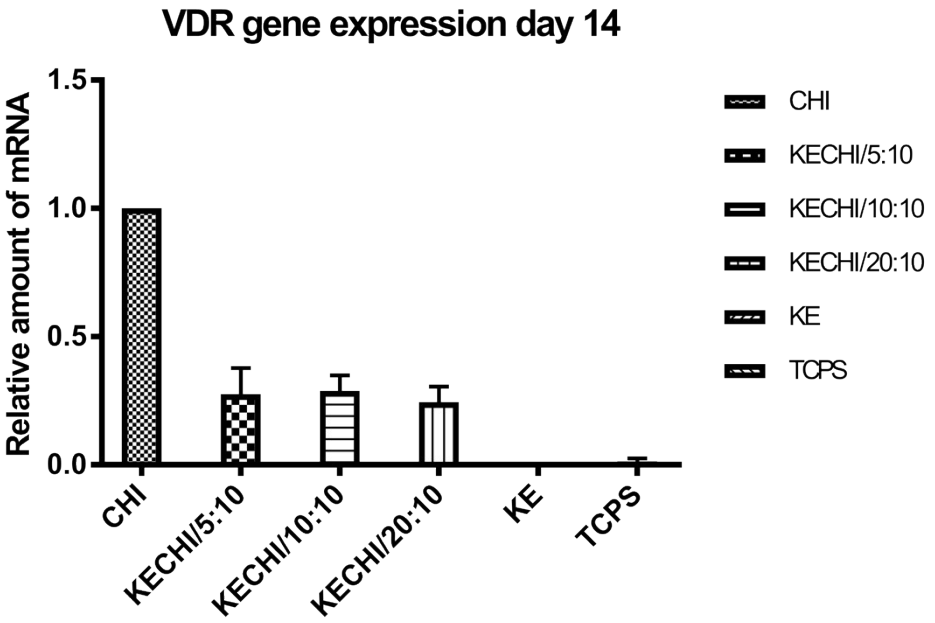
In order to visualize the difference in using osteogenic differentiation medium and normal cell culture medium when culturing human adipose stem cells (hASCs), we compare the Alizarin Red S staining of the aggregated cell sphere on day 14 (Fig. S1). The aggregated cell sphere was stained red after culturing in the osteogenic differentiation medium. On the contrary, the staining wasn't obvious when using normal cell culture medium. We presume that our KE/CHI photo-crosslinked films can enhance the cell-matrix interaction, meanwhile preserve the capability of differentiation. After the induction of chemical stimulus, the stem cells would differentiate toward the osteogenic lineage.

Fig. S1 These were the Alizarin S staining at day 14 photo of aggregated cell spheres of hASCs. The left side photo was the hASCs cultured in osteogenic differentiation medium, and the right side was cultured in normal cell culture medium.



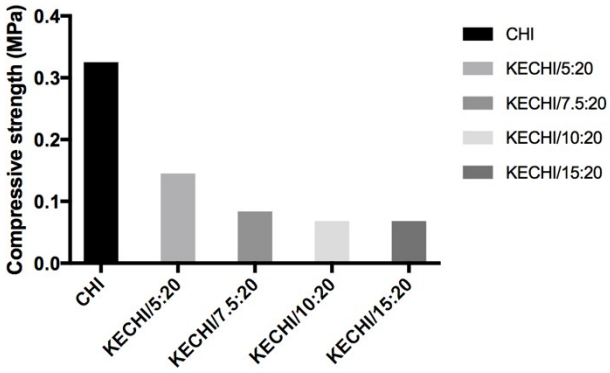
Additionally, we have examined the vitamin D receptor (VDR) gene expression on keratin/chitosan films on day 14 (Fig. S2). The relative amount of mRNA of KE/CHI-AZ films were significantly larger than of Keratin or TCPS

Fig. S2 Vitamin D receptor (VDR) gene expression on keratin/chitosan films of day 14.



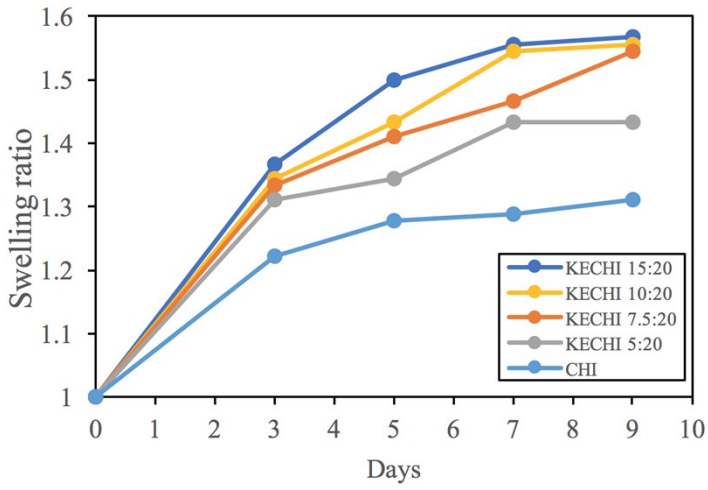
The Young's Modulus of the KE/CHI-AZ scaffolds would decrease with increasing concentration of keratin (Fig. S3). The results indirectly proved that we have successfully combine keratin with chitosan, indicating that with more azide functional group on chitosan chain conjugated with the alkyl or amine group on keratin, the self-cross-linking between chitosan would be fewer, thus decrease the mechanical strength.

Fig. S3 This was the uniaxial compression test results of KE/CHI-AZ scaffolds with different concentration of keratin content in the mixture.



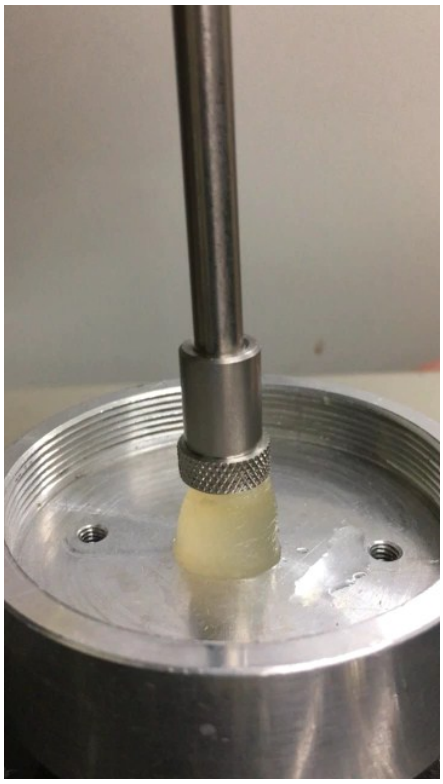
After examine the mechanical strength of the KE/CHI-AZ scaffolds, we performed the swelling ratio test (Fig. S4). Scaffolds were immersed in PBS for several time lengths. The swelling ratios were determined by comparing the diameter after soaking to the initial diameter.

Fig. S4 The swelling ratio results of KE/CHI-AZ scaffolds in PBS.



The KE/CHI-AZ scaffolds could maintain its cylindrical structure even after immersing in PBS for 21 days. The uniaxial compression test and the SEM images showed that the mechanical properties are tunable which enable us to design better scaffolds for different intended uses. The following video showed the deformation and recovery of the scaffold after compression (Video. S1). After exerting compressive force, the

scaffold would deform without wrecking. When we remove the stick gradually, the scaffold could return to its initial state and absorb almost all the water ejected during the compressing process.



Video. S1 The video of uniaxial compression test of KE/CHI-AZ scaffolds.