Engineering 3D sliding hydrogels with mobile molecular ligands to direct stem cell differentiation

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Statement of Purpose: Stem cells reside in a multifactorial environment in which they respond to various niche inputs (i.e. biochemical and mechanical) to change their fate and functions. To better decipher stem cell-niche interactions, hydrogels have been widely employed to construct artificial cell niche. Recent studies have shown that the ability of stem cells to exert traction forces and reorganize biochemical ligand clusters plays an important role in determining their differentiation. However, most hydrogels developed to date do not support mobile biochemical ligands in 3D network, thereby limiting their efficacy in promoting desirable stem cell differentiation. While physical hydrogels may offer more flexible network structure than convalently crosslinked hydrogels, the control over network stability and crosslinking density remains poor. As such, there remains a critical need to develop novel biomaterials as stem cell niche that would allow mobility of biochemical ligands within stable hydrogel network. The goals of this study are to:(1) develop sliding hydrogels crosslinkable by mobile ligands that can slide along the polymer backbone, thereby offering molecular ligand mobility; and (2) examine the efficacy of such sliding hydrogels on direct stem cell differentiation in 3D.

Methods: Alpha-cyclodextrin (αCD) based polyrotaxane (PR), the supramolecule with αCDs threaded on a polyethylene glycol (PEG) chain, was used as precursor of sliding hydrogel. PRs were made from naïve αCDs and PEG (MW 20 kDa) and then functionalized with succinic anhydride to improve aqueous solubility and vinyl sulfone to allow crosslinking and ligand incorporation. PEG (MW 6 kDa) with thiol end groups was used to crosslink the hydrogel and CRGDS peptide was used as biochemical ligand to allow cell adhesion. As a control, 4-arm PEG (MW 10 kDa) with vinyl sulfone groups and PEG with thiol groups were crosslinked as covalent hydrogels. Human adipose-derived stem cells (hADSCs) were then encapsulated in both sliding hydrogel and covalent hydrogels and cultured in different induction media and the differentiation level was examined.

Results: Our strategy allowed formation of sliding hydrogels with mobile ligands and crosslinks, thereby facilitating cells to reorganize ligands and exert traction forces in 3D hydrogel network (**Fig 1**). The coverage of αCDs on PEG chain was confirmed by ^{1}H NMR and the cover ratio was around 10%. Our sliding hydrogels supported 3D encapsulation of stem cells with high cell viability over the course of 14 days of culture in 3D, as confirmed by live/dead staining (Fig. 2). The sliding gel is characterized by After 14 days of culture in osteogenic or adipogenic medium, only sliding hydrogels, but not covalent hydrogels, induced positive osteogenesis and adipogenesis of encapsulated stem cells, as shown by staining of differentiation markers including alkaline phosphatase (osteogenesis) and Oil red O staining (adipogenesis) (**Fig. 2**).

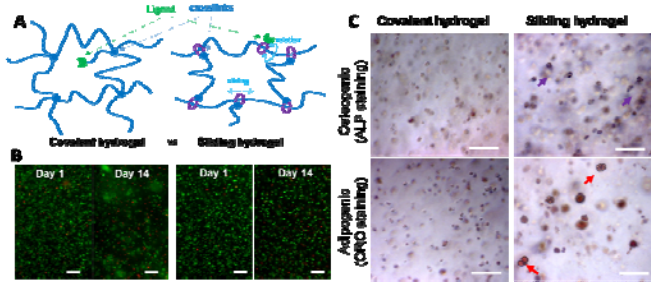

Figure 1. (A) Scheme of comparing covalent hydrogel and sliding hydrogel. **(B)** Representative micrographs of live/dead staining (day 1 and day 14, green indicates live cells while red indicates dead cells. Scale bar 100 μm). **(C)** ALP staining (day 14) and ORO staining (day 14) of hADSCs in hydrogels. (Scale bar 100 μm)

Conclusions: Here, we report a facile method for synthesizing and constructing sliding hydrogels as 3D stem cell niche with mobile biochemical ligands and crosslinks. Our sliding hydrogels support 3D encapsulation of stem cells with high cell viability, and accelerated stem cell differentiation towards osteogenesis and adipogenesis compared to conventional covalent hydrogels. Such sliding hydrogels combine the advantage of mobility of physical hydrogels and stability of covalent hydrogels, and may provide a robust biomaterials platform to enhance stem cell differentiation in 3D and facilitate mechanistic studies on deciphering the role of ligand mobility on stem cell fates and tissue regeneration.