different materials as potential candidates for anodes. Examples include Si, Sn, SnO_2, and TiO_2, among others.[3] The idea is to exploit the high capacity held by these materials. Promising initial successes have been observed. TiO_2, for instance, has already been commercialized for fast discharge-recharge applications. Nevertheless, broad utilization of these materials remains a long shot because, compared to graphite, they each face unique problems. As the race for the next generation of energy-storage application continues, Cui et al.'s work gives a new boost to the leading player of graphite.

1. Verma, P., Maire, P., and Novák, P. (2010). Electrochim. Acta *55*, 6332–6341.

2. Wu, Y.P., Rahm, E., and Holze, R. (2003). J. Power Sources *114*, 228–236.

3. Ji, L.W., Lin, Z., Alcoutlabi, M., and Zhang, X.W. (2011). Energy Environ. Sci. *4*, 2682–2699.

4. Wang, G.X., Shen, X.P., Yao, J., and Park, J.S. (2009). Carbon *47*, 2049–2053.

5. Xu, W., Wang, J.L., Ding, F., Chen, X.L., Nasybulin, E., Zhang, Y.H., and Zhang, J.-G. (2014). Energy Environ. Sci. *7*, 513–537.

6. Zheng, G., Lee, S.W., Liang, Z., Lee, H.-W., Yan, K., Yao, H., Wang, H., Li, W., Chu, S., and Cui, Y. (2014). Nat. Nanotechnol. *9*, 618–623.

7. Yan, K., Lee, H.-W., Gao, T., Zheng, G., Yao, H., Wang, H., Lu, Z., Zhou, Y., Liang, Z., Liu, Z., et al. (2014). Nano Lett. *14*, 6016–6022.

8. Kozen, A.C., Lin, C.-F., Pearse, A.J., Schroeder, M.A., Han, X., Hu, L., Lee, S.-B., Rubloff, G.W., and Noked, M. (2015). ACS Nano *9*, 5884–5892.

9. Yao, X.H., Dong, Q., Cheng, Q.M., and Wang, D.W. (2016). Angew. Chem. Int. Ed. Engl. *55*. Published online July 6, 2016. http://dx.doi.org/10.1002/anie.201601783.

10. Sun, Y.M., Zheng, G.Y., Seh, Z.W., Liu, N., Wang, S., Sun, J., Lee, H.R., and Cui, Y. (2016). Chem *1*, this issue, 287–297.

Preview

Probing the Metabolomics of Stem Cell Differentiation with Biomaterials

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In this issue of *Chem*, Ulijn, Dalby, and co-workers have developed a novel biomaterials platform as a screening tool for identifying metabolites that can bias differentiation of mesenchymal stem cells.

The fields of tissue engineering and regenerative medicine heavily rely on strategies that make use of stem cells to repair or replace damaged tissues. In order for these approaches to become widely used clinical therapies, the stem cells utilized must be easily obtained (ideally from the patient receiving treatment), triggered to differentiate into the appropriate cell types, and successfully delivered into the body either as individual cells or pre-formed into an appropriate tissue construct. To derive sufficient numbers of patient-specific stem cells, two major strategies have emerged: using adult stem cells isolated from the patient or reprogramming mature, differentiated cells from the patient into pluripotent stem cells.[1] For the repair of musculoskeletal damage, a population of adult stem cells, commonly termed mesenchymal stem cells (MSCs), holds significant therapeutic promise and is actively being investigated in numerous clinical trials. MSC-like cells can be isolated from bone marrow, adipose tissue, and the perivascular niche of patients.[2] These cells have the capacity to differentiate into bone, cartilage, adipose tissue (fat), and muscle.[2] Although MSCs can be obtained with relative ease, the efficient and scalable differentiation of these cells into desired tissues remains challenging. Biochemical methods of inducing differentiation often rely on expensive protein factors that make large-scale applications prohibitively expensive, and incomplete differentiation of stem cells can result in subpopulations of cells that undermine the function of the tissue construct and pose risks to patient health.[1] Therefore, identifying small molecules that enhance the differentiation of MSCs could markedly improve the prospects of generating engineered tissues from patient-derived stem cells.

Stem cell differentiation is directed by a combination of physical and biochemical cues. In a seminal paper published a decade ago, Discher and colleagues demonstrated that the stiffness, or elastic modulus (E), of the substrate on which MSCs were cultured biased the differentiation of these cells.[3] On relatively rigid substrates similar in stiffness to developing bone (E < 25 kPa), the cells expressed bone markers. In contrast, on intermediately stiff substrates similar to muscle (E ~ 8–17 kPa), the cells expressed

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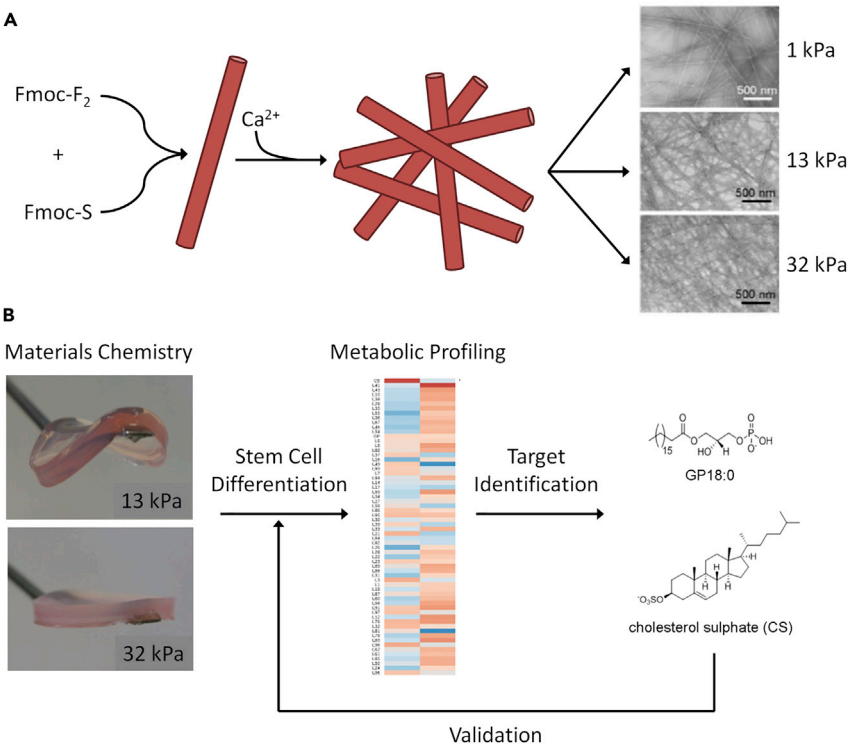

Figure 1. Using Self-Assembling Nano-fibrillar Hydrogels to Identify Changes in MSC Metabolism during Differentiation

(A) Fmoc-F_2 and Fmoc-S self-assemble into fibers that are crosslinked by calcium ions present in cell-culture medium. The stiffness of the resulting hydrogels is controlled by tuning the concentration of fibers.

(B) Hydrogel stiffness was used to direct the differentiation of MSCs into cartilage (13 kPa gels) or bone (32 kPa gels). Changes in lipid metabolism during differentiation identified GP18:0 and cholesterol sulfate (CS) as candidate supplements to enhance differentiation into cartilage and bone, respectively. The ability of GP18:0 and CS to enhance differentiation was validated with in vitro MSC culture.

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muscle markers. On soft substrates similar to brain tissue (E < 1 kPa), the MSCs expressed markers characteristic of a neuronal phenotype. Later studies encapsulating MSCs in 3D hydrogel materials that are more reminiscent of the 3D in vivo microenvironment have validated that material stiffness can bias the differentiation of MSCs, such that MSCs cultured in soft gels undergo adipogenesis to become fat cells, and MSCs cultured in stiffer gels undergo osteogenesis to become bone-forming cells.[4] Thus, a general trend has emerged that MSCs can be biased to differentiate into a desired cell type by providing an in vitro substrate with a stiffness that matches the in vivo tissue.

As stem cells differentiate, their metabolism shifts from that characteristic of the stem cells to that of the mature cell type into which the stem cell is differentiating.[5] This shift in metabolism results in changes in nutrient utilization by the cells. Changes in nutrient demand can potentially be exploited to bias cell differentiation into a desired fate by altering the availability of metabolites required for that fate. For instance, Schreiber and colleagues found that inhibition of three enzymes involved in carbon metabolism induced the differentiation of myoblasts into myotubes.[6] The major bottleneck in this approach is identifying potential target metabolites. Schreiber and colleagues utilized an RNAi-based knockdown screen to identify their target metabolic pathways,[6] a laborious and costly process. A more straightforward approach would be to induce differentiation and monitor changes in cellular metabolism over time. However, most protocols for stem cell differentiation utilize different media formulations to direct the differentiation process. Using these different media formulations makes identifying subtle changes in metabolite concentration difficult as a result of the inherent differences in the media formulations. In this issue, Ulijn, Dalby, and colleagues hypothesized that they could overcome this limitation by taking advantage of physical cues to induce MSC differentiation without altering media composition. Utilizing this novel biomaterials approach, they were able to successfully identify key metabolites involved in the differentiation of MSCs into cartilage and bone.[7]

In order to limit the bias in metabolomic analysis that could be introduced by changes in material chemistry, they employed a self-assembling fibrillar hydrogel system to control material stiffness (Figure 1A). The hydrogels are composed of two components, fluorenyl-9-methoxycarbonyl-diphenylalanine (Fmoc-F_2) and Fmoc-serine (Fmoc-S). Upon mixing in aqueous solution, the components assemble into nanoscale fibers such that Fmoc-F2 forms the core of the fibers and Fmoc-S decorates the surface of the fibers as a shell. The surfactant properties of Fmoc-S result in presentation of the polar hydroxyl and carboxylic acid groups from the surface of the fibers. The carboxylic acid groups allow the fibers to be crosslinked and stabilized by the presence of divalent cations (e.g., Ca^{2+}) in the cell-culture medium. The stiffness of the self-assembling gels is tuned simply by varying the concentrations of Fmoc-F_2 and Fmoc-S, resulting in gels with physiologically relevant elastic moduli of 1, 13, and 32 kPa. Unlike other commonly used hydrogel systems that require specific proteins or peptides to

be conjugated to the gels to facilitate cell adhesion, the nano-fibrillar gels naturally adsorb cell-adhesive proteins from the serum present in the cell-culture media. Thus, changes in stiffness can be achieved without concern for how changes in crosslinking chemistry would influence the functionalization of the gels with cell-adhesive components. The authors argue that such a system is ideal for studying stiffness-induced changes in MSC differentiation and metabolite usage, given that only minimal changes in materials chemistry are necessary for achieving a physiologically relevant range of mechanical properties.

To test this hypothesis, the authors used two MSC-type cells, classic bone-marrow-derived MSCs and perivascular stem cells (pericytes). When pericytes were cultured on the stiffest (32 kPa) gels, the cells exhibited bone markers, whereas on soft (1 kPa) gels, they expressed neural markers. On intermediately stiff (13 kPa) gels, the cells exhibited cartilage markers. Although this fits the generally accepted trend that in vivo tissue stiffness dictates in vitro MSC differentiation (i.e., bone is stiffer than cartilage, which is stiffer than neural tissue), the absence of myogenic markers on the intermediately stiff gels contrasts with earlier published results.[3] Nevertheless, Ulijn, Dalby, and colleagues have demonstrated control over MSC differentiation simply by tuning the Fmoc-F_2/Fmoc-S composition of their hydrogels. This allowed the authors to assay for lipid metabolic changes that accompany differentiation into cartilage or bone (Figure 1B). Using mass spectroscopic methods, they assessed the lipid depletion profile of the cell-culture medium for pericytes cultured on 32 kPa (osteogenic) and 13 kPa (chondrogenic) hydrogels, permitting the identification of lipid metabolites that were selectively depleted during osteogenic or chrodrogenic differentiation. After analyzing the biochemical pathways involved in the metabolism of the identified lipids, they chose two candidates for further investigation on the basis of their independent mechanisms of action: a lysophosphatidic acid (GP18:0) for chondrogenesis and cholesterol sulfate (CS) for osteogenesis.

To determine whether the identified metabolites were capable of controlling the differentiation of both bone-marrow-derived MSCs and pericytes, the authors cultured these cells with either GP18:0 or CS supplemented in the medium. As predicted, the addition of GP18:0 resulted in increased expression of cartilage markers in both pericytes and MSCs, similar to treatment with chondrogenesis-induction medium. Similarly, treatment with CS resulted in increased expression of bone markers in both cell types, as did treatment with standard osteogenesis-induction medium. As a demonstration of the therapeutic potential of these results, pericytes were encapsulated within the stiff (32 kPa) hydrogels and injected into tissue-mimicking collagen gels. After 28 days in culture, tissue mimics that were treated with CS exhibited enhanced mineralization, suggesting that treatment with CS can enhance the bone-forming capacity of transplanted stem cells. Furthermore, treatment with CS resulted in significant mineralization in all samples, indicating more homogeneous control over differentiation than in controls. Such homogeneous differentiation is crucial for the safe and reproducible implementation of stem cell therapies.[1]

The work by Ulijn, Dalby, and colleagues represents a growing trend toward using libraries of materials to screen for cellular responses. Although it is commonplace to screen large libraries of small molecules or proteins for desired therapeutic efficacy, only more recently have researchers begun to screen libraries of biomaterials for desired effects.[8] Although Ulijn, Dalby, and colleagues used a relatively small library of three hydrogels with different levels of stiffness, they were able to elucidate novel metabolic targets to direct the differentiation of stem cells.[7] Aided by automated synthesis and robotic handling systems, the preparation of larger libraries of biomaterials can be achieved, and these have the potential to uncover combinatorial effects of material properties on stem cell behavior.[8] Such an approach has been used previously to identify properties that are critical for hematopoietic stem cell expansion in vitro and result in enhanced stem cell engraftment in vivo.[9] More recently, novel materials-chemistry approaches have been used to generate hydrogel arrays with variable stiffness and cell-adhesive ligand presentation, and these arrays have revealed that these two parameters interact to modulate MSC spreading and proliferation.[10] Looking forward, utilizing material platforms to identify conditions optimal for stem cell expansion and differentiation has the potential to reduce the cost and improve the safety and efficacy of regenerative medicine therapies.

1. Trounson, A., and McDonald, C. (2015). Cell Stem Cell *17*, 11–22.

2. Charbord, P. (2010). Hum. Gene Ther. *21*, 1045–1056.

3. Engler, A.J., Sen, S., Sweeney, H.L., and Discher, D.E. (2006). Cell *126*, 677–689.

4. Huebsch, N., Arany, P.R., Mao, A.S., Shvartsman, D., Ali, O.A., Bencherif, S.A., Rivera-Feliciano, J., and Mooney, D.J. (2010). Nat. Mater. *9*, 518–526.

5. Folmes, C.D.L., Dzeja, P.P., Nelson, T.J., and Terzic, A. (2012). Cell Stem Cell *11*, 596–606.

6. Bracha, A.L., Ramanathan, A., Huang, S., Ingber, D.E., and Schreiber, S.L. (2010). Nat. Chem. Biol. *6*, 202–204.

7. Alakpa, E.V., Jayawarna, V., Lampel, A., Burgess, K.V., West, C.C., Bakker, S.C.J., Roy, S., Javid, N., Fleming, S., Lamprou, D.A., et al. (2016). Chem *1*, this issue, 298–319.

8. Yang, F., Mei, Y., Langer, R., and Anderson, D.G. (2009). Comb. Chem. High Throughput Screen. *12*, 554–561.

9. Lutolf, M.P., Doyonnas, R., Havenstrite, K., Koleckar, K., and Blau, H.M. (2009). Integr Biol (Camb) *1*, 59–69.

10. Le, N.N.T., Zorn, S., Schmitt, S.K., Gopalan, P., and Murphy, W.L. (2016). Acta Biomater. *34*, 93–103.