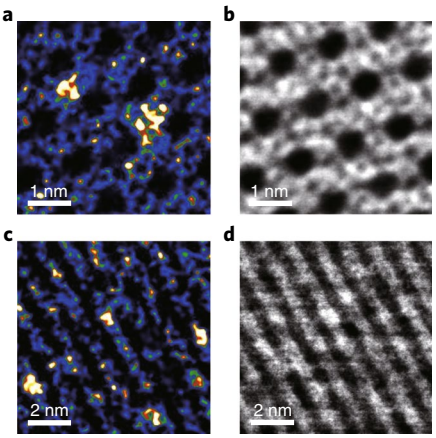

Fig. 2 | Identification of the location of sub-nanometric Pt clusters within the zeolite MFI structure. a,**b**, Large-area and detailed HR HAADF-STEM image (**a**) and the corresponding iDPC image (**b**) of a K-PtSn@MFI catalyst in the [010] orientation. **c**,**d**, Images of the same material in the tilted-[010] orientation. Figure reproduced from ref. [4], Springer Nature Ltd.

potential provided it can be applied to other zeolite framework structures. A zeolite material with a similar, yet distinct framework structure is MEL, which also contains two 10MR channels. The two types of 10MR channels of MEL are both straight, leading to two distinct intersections, one being large and another being small. It would be very interesting to explore if, by playing with the interfacial chemistry between the OSDA and the zeolite framework, we can selectively place sub-nanometric metal particles in one intersection space, but not in the other intersection space. If this indeed can be done for MEL, we are not limited to other 10MR zeolite frameworks, such as MWW, and can extend the approach to 8MR and 12MR zeolite frameworks, such as CHA or AFI. Interestingly, certain OSDAs, such as tetraethyl ammonium (TEA^+), are very versatile in steering the synthesis. For example, TEA^+ can either form an 8MR (CHA) or 12 MR zeolite framework (AFI) from the same synthesis gel[6]. One could then imagine that it should become feasible to selectively trap sub-nanometric metal particles in one zeolite cage or channel (for example, 8MR) and at the same time keep another zeolite cage or channel (for example, 12MR) completely free so that reactants and reaction products can freely move forward and backward to the catalytically active metal nanoparticle. If such concepts work, we would be entering a new era in the rational design of zeolite-based catalysts. The work of Corma and co-workers illustrates with the MFI showcase that such an approach should become feasible in the years to come. ❐

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STEM CELLS

Mastering their own fates through the matrix

With their ability to give rise to many different cell types, stem cells have long been a target of scientists who seek to achieve control over their differentiation. New evidence suggests that stem cells influence their own fates through protein deposition and physical remodelling of their microenvironment.

Eric L. Qiao, Sanjay Kumar and David V. Schaffer

It has long been known that stem cells are influenced by the local physical and biochemical cues present in their niches. Engineered biomaterials direct stem cell differentiation by controlling the presentation of these cues to stem cells. However, an often overlooked factor in studies of stem cell differentiation is the deposition of extracellular matrix (ECM) proteins by the stem cells themselves. To better understand how nascent protein deposition influences the differentiation of human mesenchymal stromal cells (MSCs), Jason Burdick and colleagues[1] report in *Nature Materials* visualization of protein deposits in a three-dimensional (3D) material and show that MSC mechanosensing is dramatically altered through interactions with this protein layer.

The stiffness, viscoelastic properties and topography of the ECM are all known to play important roles in controlling stem cell fate[2,3]. In addition to these properties, cells secrete and deposit ECM proteins over time, so their local microenvironment has the potential to change drastically independent of the properties of their matrix. To visualize these changes, Burdick et al. employed bio-orthogonal non-canonical amino acid tagging (BONCAT) to replace naturally occurring methionine residues in proteins with azidohomoalanine (AHA). The azide group in AHA could then be conjugated with a cyclooctyne-bearing fluorophore to visualize the secreted proteins. Applying this labelling technique to MSCs cultured in a variety of 3D hydrogel systems, the researchers found that a secreted protein layer formed in all culture platforms within one day of culture. Further imaging showed that focal adhesions co-localized with deposited proteins, suggesting direct interaction between cells and deposited ECM proteins. Over the course of six days in culture, the protein deposit formed into a mesh-like structure and continued to grow in thickness. Prior studies have shown that MSCs make key fate commitment decisions early, probably within the first 24 hours in culture in the presence of differentiation cues[4], so it is significant that detectable levels of protein secretion were observed within this window.

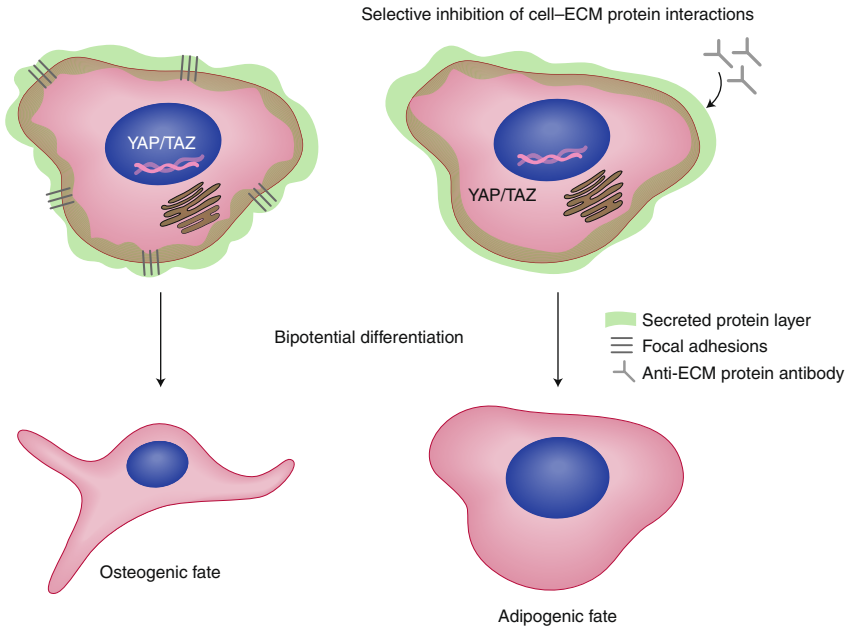

Fig. 1 | The role of ECM deposition and matrix remodelling in mesenchymal stromal cell (MSC) differentiation. When cultured in 3D hydrogels, MSCs deposit a layer of ECM proteins including fibronectin and collagen as well as remodel the underlying hydrogel matrix. ECM deposition and matrix remodelling, in turn, affect the localization of transcriptional regulators YAP/TAZ and bias downstream differentiation.

Next, the authors set out to determine the interaction between deposited ECM proteins and known signalling pathways by using blocking antibodies to inhibit these interactions (Fig. 1). When cells were treated with collagen and fibronectin-specific antibodies, they found a significant reduction in cell spreading as well as in the growth rate of the deposited protein layer over six days. Additionally, inhibition of the cell-secreted fibronectin interactions changed the behaviour of known adhesive signalling pathways. In particular, blocking of interactions with secreted fibronectin shifts the localization of YAP/TAZ, normally localized to the nucleus in MSCs, to a more cytoplasmic distribution. YAP/TAZ is a known upstream regulator of fate commitment, and nuclear localization of YAP/TAZ is associated with osteogenic differentiation[5,6]. With this in mind, the authors assessed MSC differentiation and found that in untreated cells, osteogenic differentiation was the favoured lineage. When cell–ECM interactions were treated with soluble RGD or blocking antibodies, MSCs preferentially differentiated into an adipogenic phenotype. Together, these results suggest that nascent protein deposits inform MSC fate commitment through YAP/TAZ mechanosensing.

Interestingly, protein deposit thickness was influenced by matrix stiffness, suggesting that the ability of the matrix to restrict cell spreading plays a role in regulating protein deposition. Using a viscoelastic, hyaluronic acid (HA)-based hydrogel system, the authors were able to visualize a deposited ECM layer as early as four hours in culture. Treatment of cells with either a protein secretion inhibitor or matrix metalloproteinase (MMP) inhibitor decreased both YAP/TAZ nuclear to cytoplasmic ratio and relative amounts of osteogenic differentiation compared to untreated cells. Taken together, these results suggest that both nascent protein deposition as well as metalloproteinase-mediated remodelling of the matrix are important in regulating stem cell fate commitment in viscoelastic environments.

Burdick and colleagues' study demonstrates the importance of stem cell-derived ECM cues in fate commitment and provides insight into how stem cells can provide their own differentiation cues through nascent protein deposition. In particular, it underscores the importance of considering not only the material properties but also the effect of secreted ECM components when interpreting results from studies performed in engineered materials. For example, the authors' findings that initial hydrogel stiffness influences the formation of the secreted protein layer suggest that previously observed stiffness-dependent differences in stem cell behaviour may be due to indirect influences on protein deposition rather than through direct cell-biomaterial interactions. This work also demonstrates that the secretome can be a potent tool in engineering biomaterials for specific applications. In fact, knowledge of the MSC secretome coupled with protein capture of secreted factors is already being used to generate tailor-made scaffolds that could be used in tissue engineering or regenerative medicine[7].

Beyond the potential practical applications, this work also identifies several potential future directions. For instance, it demonstrates the importance of key secreted ECM proteins such as fibronectin, yet we know that the secretome consists of a complex network of proteins. Although this work suggests that perturbations to a small number of these protein interactions can change cell behaviour, it is unknown to what extent the collective composition of the secretome is important. Additionally, the results demonstrated in three dimensions beg the question of how the secretome properties change in 2D culture platforms. Whether these results are applicable in 2D may have an impact on the interpretation of prior 2D results. Finally, the observed similarities in cell behaviour when treated with either MMP inhibitors or protein secretion inhibitors suggest that MMP-mediated turnover of secreted ECM proteins plays a role in directing cell behaviour. A dynamic secretome would be fascinating to study in the context of major cellular changes such as differentiation. The authors' work in this field represents a major step forward in understanding the interactions between stem cell and secretome as well as an exciting new lens with which to study stem cell behaviour. ❐

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