retraction force, the single bond between α5β1 in a live fibroblast and a fibronectin fragment switched from a catch to a slip bond — that is, from a bond whose lifetime increases with force to a bond whose lifetime decreases with force, which had been shown for purified proteins[9]. This behaviour is dependent on fibronectin's Arg-Gly-Asp (RGD) tripeptides, the specific sites recognized by α5β1 integrins, reinforcing the finding that integrin biphasic force response is mainly determined by α5β1 integrins. The authors also studied if intracellular partners of activated integrins were involved in this fast force response. Focal adhesion kinase (FAK) and the Src-kinase family are some of the first downstream molecules that interact with integrin tails for signalling. Inhibition of FAK activity or the Src-kinase family with specific pharmacological agents abolished the biphasic force response, suggesting that these are involved in this process.

The work of Müller and co-workers reveals that a rapid integrin mechanosensing mechanism is operative in live fibroblasts but several questions remain unanswered. Studies with recombinant integrins suggest that catch–slip bond behaviour can be observed in a single purified α5β1 molecule[9], whereas in a living cell FAK and Src-kinase family activities also seem to be required. This difference may arise from the activation of additional integrins or the strengthening of already activated integrins. Future studies assessing whether FAK and Src activities are required for the catch–slip bond behaviour in cells expressing a single α5β1 integrin would aid our understanding of this process. It would also be informative to determine if FAK and Src are activated by force before the 0.5-s strengthening response occurs, since the exact time frame at which FAK is activated by mechanical force on fibronectin-coated substrates[10] is not known and Src is activated within 0.3 s after force is applied to integrins[11]. Furthermore, whereas it is known that that phosphatidylinositol 4,5-biphosphate (PIP_2) regulates FAK activity[10], Müller and colleagues found that PIP_2 had no effect on the force response of α5β1 integrins but the reason why remains to be resolved.

Regardless of the underlying mechanism, the finding that integrin mechanosensing is essentially instantaneous constitutes a significant step in establishing integrins as first responders in mechanosensing. The work of Müller and colleagues will stimulate researchers in the fields of mechanobiology and mechanomedicine to explore in detail how various integrin subtypes respond to force and how integrins, in crosstalk with cell–cell mechanosensor cadherins[12], regulate cell adhesion, migration and invasion in embryogenesis, physiology and cancer. ❐

Ning Wang is in the Department of Biomedical Engineering, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan, Hubei 430074, China, and the Department of Mechanical Science and Engineering, College of Engineering, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801, USA.
e-mail: nwangrw@illinois.edu

References

1. Tamkun, J. W. *et al. Cell* **46,** 271–82 (1986).
2. Pierschbacher, M. D. & Ruoslahti, E. *Nature* **309,** 30–33 (1984).
3. Wang, N., Butler, J. P. & Ingber, D. E. *Science* **260,** 1124–1127 (1993).
4. del Rio, A. *et al. Science* **323,** 638–641 (2009).
5. Grashoff, C. *et al. Nature* **466,** 263–266 (2010).
6. Strohmeyer, N., Bharadwaj, M., Costell, M., Fässler, R. & Müller, D. J. *Nat. Mater.* **16,** 1262–1270 (2017).
7. Taubenberger, A. *et al. Mol. Biol. Cell* **18,** 1634–1644 (2007).
8. Gingras, A. R. *et al. EMBO J.* **27,** 458–469 (2008).
9. Kong, F., García, A. J., Mould, A. P., Humphries, M. J. & Zhu, C. *J. Cell Biol.* **185,** 1275–1284 (2009).
10. Seong, J. *et al. Proc. Natl Acad. Sci. USA* **110,** 19372–19377 (2013).
11. Na, S. *et al. Proc. Natl Acad. Sci. USA* **105,** 6626–6631 (2008).
12. le Duc, Q. *et al. J. Cell Biol.* **189,** 1107–1115 (2010).

MATRIX DEGRADATION

Making way for neural stemness

The influence of matrix stiffness and degradation on neural progenitor cell stemness was investigated in a three-dimensional culture system, highlighting the role of remodelling in enhancing cell-to-cell interaction and ultimately maintaining neural stemness.

Phillip H. Kang, Sanjay Kumar and David V. Schaffer

Stem cells share two distinguishing characteristics: self-renewal to maintain an undifferentiated population, and the capacity or potency to differentiate into one or more cell types. Moreover, the proper maintenance of these defining properties is critical for organogenesis during development, continued tissue function throughout adulthood, and the promise of cell-replacement therapies. Biomaterials have been progressively applied to address several needs in this field. In particular, approaches to deliver stem cells for regenerative medicine applications have historically suffered from limited cell survival, retention and functional differentiation at the site of implantation, and the use of biomaterials as cell delivery vehicles may improve such outcomes. In parallel, these materials can yield further basic insights into the impact of the microenvironment or niche on stem cell function. Towards these collective goals, Sarah Heilshorn and colleagues[1] now report in *Nature Materials* that the maintenance of neural progenitor cell (NPC) stemness in three-dimensional (3D) culture strikingly depends on the cells' ability to degrade or remodel their surrounding matrix, which allows cells to establish contact with their cellular neighbours and thereby trigger intracellular signals that maintain a stem-like identity.

Within their niche, stem cells are regulated by a variety of extracellular cues, including mechanical inputs such as matrix stiffness, topography and stretch. To date, research exploring the effects of such mechanical cues on NPCs has primarily been conducted on two-dimensional (2D) substrates[2,3], and while some work has been pursued in three dimensions[4], the extent to which 2D findings apply in three dimensions in general — as well as the mechanisms novel to 3D contexts — are unclear. Notably, in three dimensions, cell-secreted proteases can degrade and remodel the surrounding matrix, and matrix degradation has been shown to significantly influence stem cell behaviour in other systems[5]. However, the consequences of this dynamic process on NPC behaviour have not yet been thoroughly investigated.

Heilshorn and colleagues engineered a material composed of alternating elastin-like and bioactive domains, wherein the elastin-like regions served as the structural backbone, and the bioactive domains contained cell-adhesive peptide motifs and, importantly, were sensitive to proteolytic cleavage. By modulating the protein weight percentage and crosslinking density, the researchers could individually tune the stiffness and degradability of the resulting gels. Using this material design, they found that although expression of NPC stemness markers nestin and Sox2 did not correlate with initial gel stiffness (0.5 to 50 kPa), they increased with increased degradability. In addition to marker expression, the researchers demonstrated that degradability enhanced NPC self-renewal and potency, the defining functional properties of stem cells. Numerous prior studies have reported that stiffness does impact NPC behaviour, including self-renewal and differentiation, and the discrepancy between these earlier reports and this study could potentially be attributed to the material used. Regardless, the researchers provide clear evidence for the role of degradability in NPC maintenance in this system.

The researchers then tested whether the observed findings were mediated by integrin signalling and cytoskeletal contractility, as described previously for mesenchymal stem cells (MSCs) in 3D degradable gels[5]. However, it was evident that neither small molecule activators/inhibitors of cytoskeletal contractility, nor function-disrupting mutations of the cell-adhesive motifs affected degradation-dependent NPC maintenance, indicating that degradability maintains NPC stemness through a mechanism independent of integrin-mediated tension sensing. The researchers suggested the difference between the two cell types may be attributed to the less contractile nature of NPCs compared to MSCs, such that a cell's intrinsic mechanical properties may tune how biophysical cues regulate cellular behaviour.

The key premise of this study is instead that matrix degradation allowed for increased cell–cell contact, which maintained NPC stemness through transcriptional regulation (Fig. 1). The researchers showed this using an engineered elastin-like protein material platform, but they also extended the finding to other platforms to assess generality. Specifically, within a second material — a chemically crosslinked PEG hydrogel sensitive to cleavage by an enzyme disintegrin and metalloprotease 9 (ADAM9) — they also found that nestin and Sox2 expression increased as the percentage of cleavable crosslinks was increased at a given stiffness, while stiffness did not have a significant effect within the narrow range tested for this platform (600–1,800 Pa). These results further illustrate the finding that degradability is important, though a larger stiffness range would rule out a potential mechanical effect in this system. As a third material system, the researchers used a physically crosslinked alginate hydrogel, which facilitated consistent expression of nestin and Sox2. By contrast, inhibiting matrix modification by introducing covalent crosslinks into the alginate material inhibited cell spreading and resulted in decreased nestin and Sox2 expression.

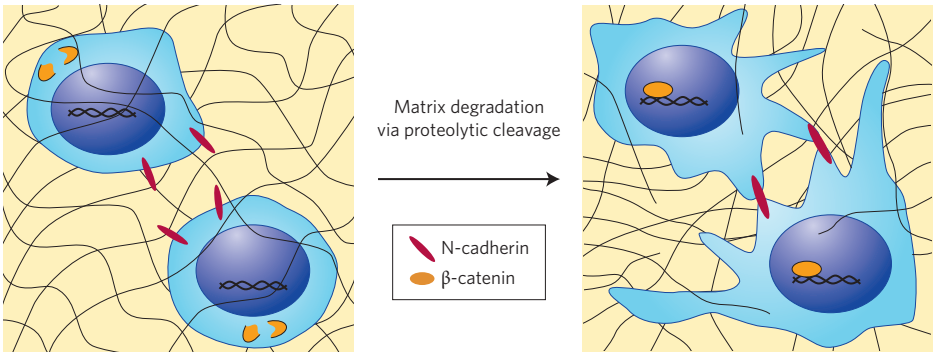

Figure 1 | The role of matrix degradation in neural progenitor cell stemness. In 3D culture systems, matrix degradation via ADAM9, an enzyme disintegrin and matrix metalloprotease, enables neural progenitor cells (NPCs) to establish N-cadherin-based cell–cell contact. This activates the Wnt signalling pathway effector, β-catenin, which acts as a transcriptional co-activator of genes that maintain NPC stemness.

The researchers then mechanistically investigated how matrix degradability could affect NPC maintenance and found that knockdown of ADAM9 reduced hydrogel degradation and nestin/Sox2 expression in three dimensions, but not in 2D controls. They then assessed whether changes in cell–cell contact, which would be enabled by matrix degradation, could influence stemness maintenance. Supporting this hypothesis, inhibition of N-cadherin reduced nestin/Sox2 expression in highly degradable hydrogels. Furthermore, increasing cell density within less degradable hydrogels, which presumably led to more cell–cell interactions, moderately increased marker expression. The researchers considered whether N-cadherin engagement played a role in maintaining NPC stemness through its known interaction with β-catenin signalling and found that high degradability promoted β-catenin stabilization, activation and transcriptional activity. Some studies have shown that Wnt/β-catenin signalling promotes neuronal differentiation of NPCs[6], whereas others have found a role in promoting self-renewal[7], and these conflicting roles for β-catenin are apparently modulated by other signals the NPC receives as well as which downstream transcriptional targets β-catenin regulates[8]. This study reported that inhibition of N-cadherin decreased expression of β-catenin responsive gene Axin2, and further investigation may elucidate how and why Wnt/β-catenin signalling favours self-renewal in this system.

The stem cell and materials science fields are increasingly appreciating how biomaterials can be applied as tools for both basic investigation and biomedical translation. In this study, Heilshorn and colleagues describe how NPC stemness can be maintained by 3D matrix degradability. The work also highlights several potential future directions. For instance, the study concludes that cell–cell contact exerts important effects on NPC behaviour. However, *in vivo* investigations in the hippocampus show that NPC clonal populations remain in close proximity within a niche for extended periods of time[9], and it thus remains to be seen whether matrix degradability limits cell–cell association *in vivo*, or in fact serves as a means to investigate the importance of cell–cell contact *in vitro*. In addition, a number of prior studies have illustrated the importance of matrix stiffness in regulating self-renewal and differentiation — both in two dimensions and increasingly in three dimensions with physically crosslinked, non-degradable gels — and the relationship between degradability, stiffness and intracellular signalling will be a rich area for investigation. Finally, in general, matrix metalloproteinases (MMPs) are known to play an important role in central nervous

system development, plasticity and repair *in vivo*. Therefore, understanding how such MMP-mediated remodelling modulates both biochemical and biophysical extracellular matrix properties to impact cell behaviour will offer further insights into cell regulation in both physiological and pathological conditions. The advances described in this work thus promise to unlock additional future answers to how matrix regulates NPC biology *in vitro* and *in vivo*. ❐

Phillip H. Kang, Sanjay Kumar and David V. Schaffer are in the Department of Bioengineering and the Department of Chemical and Biomolecular Engineering, University of California, Berkeley, California 94720, USA.
e-mail: skumar@berkeley.edu; schaffer@berkeley.edu

References
1. Madl, C. M. *et al. Nat. Mater.* **16,** 1233–1242 (2017).
2. Saha, K. *et al. Biophys. J.* **95,** 4426–4438 (2008).
3. Rammensee, S., Kang, M. S., Georgiou, K., Kumar, S. & Schaffer, D. V. *Stem Cells* **35,** 497–506 (2017).
4. Banerjee, A. *et al. Biomaterials* **30,** 4695–4699 (2009).
5. Khetan, S. *et al. Nat. Mater.* **12,** 458–465 (2013).
6. Lie, D.-C. *et al. Nature* **437,** 1370–1375 (2005).
7. Chenn, A. & Walsh, C. A. *Science* **297,** 365–369 (2002).
8. Qu, Q. *et al. Mol. Cell. Biol.* **33,** 2551–2559 (2013).
9. Bonaguidi, M. A. *et al. Cell* **145,** 1142–1155 (2011).

DISEASE MODELS

Method in the madness of fibrosis

Blocking the growth of new blood vessels has been shown to alter fibrosis in livers in a disease stage-specific manner. *In vitro* models of fibrosis were developed to understand this process, highlighting the role of environmental mechanics.

Geoffrey C. Gurtner and Jagannath Padmanabhan

Tissue fibrosis in response to injury can affect organs such as the lungs and the liver, and often results in organ failure and death[1]. Recent advances in fibrosis research have revealed that mechanical cues such as physical properties of the tissue and mechanical forces can also contribute to fibrosis in a process called mechanotransduction[2,3]. Now, writing in *Nature Materials*, Yanan Du and colleagues[4] have developed novel *in vitro* models to mimic fibrotic liver tissue and utilized them to understand how mechanical cues affect fibrosis, the role of angiogenesis in fibrosis and the response to anti-angiogenic drugs.

Fibrosis is initiated by injury or toxin-mediated tissue damage, which induces an aggravated inflammatory response[5]. Increased inflammation is accompanied by abnormal new vessel growth and the activation of scar-producing myofibroblast cells. The end result is the replacement of injured tissue with a patch of dysfunctional scar, which is primarily composed of myofibroblasts and collagen[5]. While several cellular and biomolecular mediators of fibrosis have been identified, the development and progression of fibrosis at the organ level remains poorly understood. Repair of injured tissue varies considerably between species and organisms, and it is now known that injured tissue can be fully regenerated in some organisms, whereas in human adults, formation of fibrotic or scar tissue is prevalent. Despite the many years of research in fibrosis, effective clinical treatment strategies to prevent or reduce scar tissue remain elusive.

Studies confirming the presence of abnormal blood vessel growth in fibrotic tissues date back to the 1960s[6]. Since then the role of angiogenesis in fibrosis progression, particularly in the lung and the liver, has been confirmed by multiple research groups[7,8]. Based on these findings, anti-angiogenesis drugs were enthusiastically embraced for the treatment of liver fibrosis[7]. Unfortunately, in-depth examination of their efficacy has produced conflicting results. Blocking angiogenesis has been shown to reduce fibrosis in some cases, but there is also evidence that indicates that anti-angiogenesis therapy might be ineffective or even detrimental to the resolution of fibrosis[8,9]. These observations underscore the frustrating unpredictability of fibrosis treatments and the challenges in developing reliable new therapies. Du and colleagues show that there is indeed a method to the madness, since angiogenesis therapy works

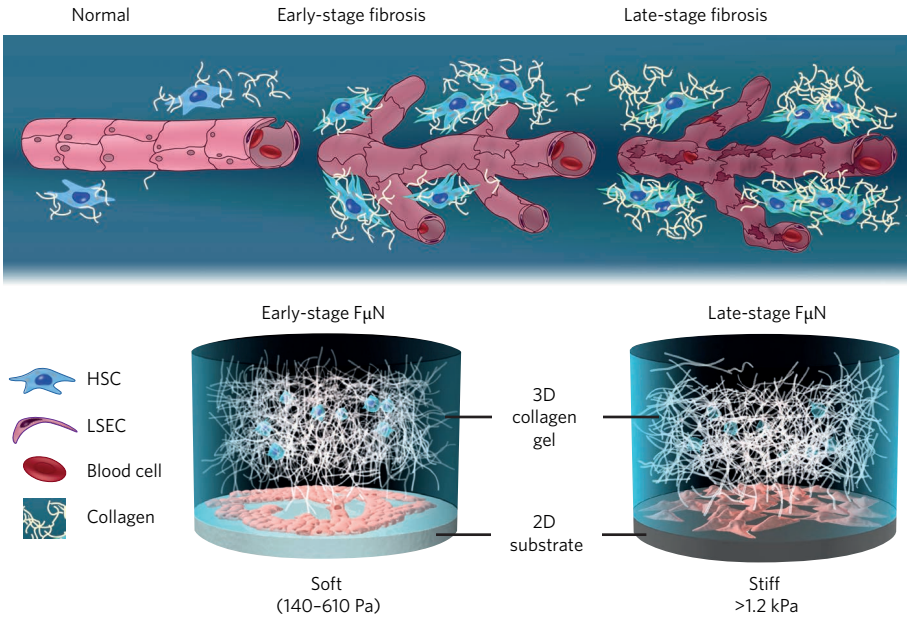

Figure 1 | Biomimetic *in vitro* models of liver fibrosis. **a**, Schematic demonstrating the stages of liver fibrosis that highlights the differences in blood vessel morphology and collagen fibre content. **b**, *In vitro* models of liver fibrosis were developed to recapitulate early and late stages of the disease. These models (microniches) were composed of LSECs cultured on mechanically distinct (soft and stiff) substrates overlaid with a collagen hydrogel containing HSCs. Reproduced from ref. 4, Macmillan Publishers Ltd.