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Review Article

Scaffold Stiffness and Lineage Commitment in Human Mesenchymal Stromal Cells

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Abstract

Background: Matrix elasticity was reported in 2006 to direct mesenchymal lineage choice, and became one of the most cited claims in tissue engineering.

Objective: To review how that claim has held up, what the objections are, and whether elastic modulus is the right variable.

Methods: Narrative review of the founding studies, the mechanotransduction literature, and the work that has qualified them.

Findings: Reported windows do not agree between systems: osteogenesis at 25-40 kPa in two dimensions, 11-30 kPa in three-dimensional alginate, and 17 kPa where stress relaxation is fast. Stiffness had no effect on polydimethylsiloxane across four orders of magnitude, attributed to ligand tethering rather than elasticity. The same gel yields different moduli by measurement method.

Conclusions: The direction of the effect reproduces; the mechanism does not. Modulus is best treated as a proxy rather than the causal variable.

Keywords: matrix stiffness; mechanotransduction; mesenchymal stromal cells; viscoelasticity; hydrogels

Introduction

That cells respond to the mechanical properties of what they are grown on was established before it was applied to differentiation: locomotion and focal adhesions are regulated by substrate flexibility [1], morphology and cytoskeletal structure follow substrate stiffness [2], and cell shape and cytoskeletal tension regulate mesenchymal lineage commitment through RhoA [3]. The framing that tissue cells feel and respond to substrate stiffness gathered these observations into a principle [4].

The claim that made the field was narrower and more striking: on polyacrylamide gels matched to the elasticity of brain, muscle and bone, naive mesenchymal cells committed to neurogenic, myogenic and osteogenic lineages respectively [5]. Soft matrices at 0.1–1 kPa were neurogenic, 8–17 kPa myogenic and 25–40 kPa osteogenic, with lineage markers upregulated five-, six- and fourfold, and blebbistatin abolishing specification (under twofold different from naive cells). The role of stiffness in regulating cell behaviour was reviewed shortly after [6].

Two decades on, the direction of that result reproduces widely and the mechanism is contested. This review is about the contest.

Materials and Methods

Records were retrieved from Crossref, OpenAlex and publisher pages for the terms *matrix stiffness*, *mechanotransduction*, *mesenchymal stem cell*, *hydrogel*, *stress relaxation* and *YAP*. Priority was given to studies that varied stiffness against a controlled confound, and to work that qualified or challenged the founding result. Every bibliographic record was confirmed at source, and quantitative values were taken from the source rather than from secondary citation.

The machinery

How a cell converts substrate mechanics into transcription is better understood than whether modulus

is what it senses. Force is transmitted from the extracellular matrix to the nucleus through a physically continuous linkage [7], integrins act as the biomechanical sensors at the interface [8], and a molecular clutch model describes how force transmission depends on rigidity [9]. YAP and TAZ are the transcriptional relays [10,11], and their nuclear entry is triggered by force acting on nuclear pores [12]. Nuclear lamin-A scales with tissue stiffness and enhances matrix-directed differentiation [13]. The role of mechanical force in stem cell behaviour has been reviewed across development and regeneration [14].

None of this settles what the cell is measuring. A clutch responds to the rate at which adhesions load, which depends on ligand anchoring as much as on bulk modulus.

The tethering objection

The most substantial challenge came in 2012. Across polydimethylsiloxane substrates spanning four orders of magnitude, cell spreading and differentiation were unaffected by stiffness; on polyacrylamide, cells on gels of low modulus could not form stable focal adhesions. The interpretation was that polyacrylamide results reflect how the collagen is anchored to the gel rather than the gel's elasticity, so extracellular-matrix tethering, not stiffness, regulates fate [15].

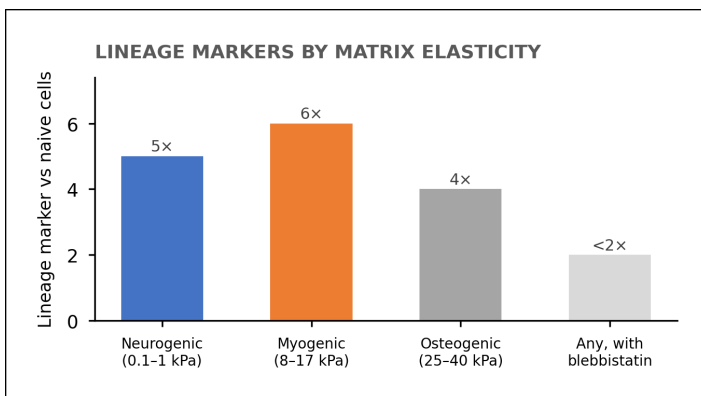


Figure 1: Lineage marker upregulation on matched-elasticity gels, with the blebbistatin control. Inhibiting non-muscle myosin abolishes specification, which is the internal evidence that the effect is contractility-dependent. Data from Engler et al. [5].

The rebuttal came from the original laboratory two years later, in the same journal. Varying porosity and linker density substantially altered tethering without altering differentiation, and the interplay of stiffness and protein tethering was resolved in favour of stiffness [16]. Nanoscale spatial organisation of adhesive ligands was subsequently shown to direct fate alongside stiffness [17].

Both papers remain cited approvingly. The field has not adjudicated between them, and a review that cites only one is choosing a side without saying so.

Three dimensions, where nothing varies alone

In three-dimensional culture the confounds are structural rather than incidental. Traction-mediated manipulation of the cell-matrix interface controls fate in alginate, with osteogenesis predominating at 11–30 kPa [18] — an overlapping but not identical window to the two-dimensional one. In covalently crosslinked hydrogels, degradability rather than modulus directs fate through cellular traction, and fate stopped correlating with cell morphology [19]. Volume expansion and TRPV4 activation regulate fate in three dimensions by a further route [20]. The practical difficulties of using hydrogels for cell culture have been set out for the field [21].

Viscoelasticity is the variable this exposed. Substrate stress relaxation regulates spreading independently of initial modulus [22], and in hydrogels with tunable relaxation, cells formed a mineralised collagen-rich matrix at an initial elastic modulus of 17 kPa when relaxation was fast [23] — a modulus that the two-dimensional literature calls myogenic. The effects of matrix viscoelasticity on cellular behaviour have since been reviewed comprehensively [24].

Memory and dose

Exposure history matters as well as instantaneous stiffness. Hydrogels stiffened in situ probe short- and long-term responses [25], and mechanical memory and dosing were shown to influence fate: cells carry a record of prior substrate stiffness that biases later commitment [26]. Extended exposure to stiff microenvironments produces persistent chromatin remodelling [27], which offers a physical basis for the memory.

Measurement

A quieter problem underlies all of the above. Comparing methods on the same hydrogels, up to a two- to threefold variation in elastic modulus is observed across the same rigidity gradient, with atomic force microscopy and micropipette aspiration systematically returning smaller values because they probe the surface rather than the bulk [28]. Protocols for measuring soft surfaces and hydrogels by atomic force microscopy have been standardised for exactly this reason [29].

If a 10 kPa gel in one paper is not the same object as a 10 kPa gel in another, the disagreement between reported windows is partly arithmetic rather than biological.

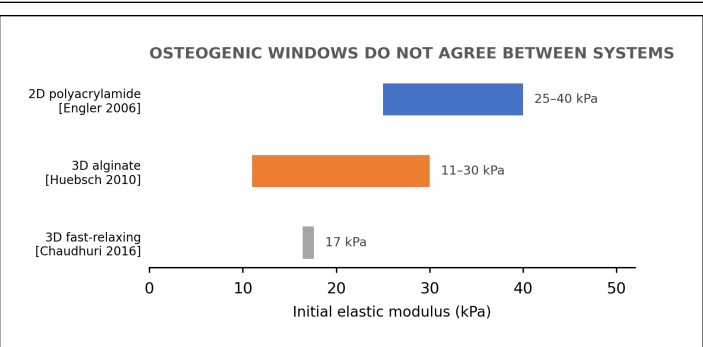


Figure 2: Elastic modulus windows reported for osteogenesis in three different systems. The windows overlap but do not agree, and the fast-relaxing gel produces bone-like matrix at a modulus the two-dimensional literature assigns to muscle. Data from Engler et al. [5], Huebsch et al. [18] and Chaudhuri et al. [23].

Discussion

Three conclusions follow. First, the direction of the effect is robust: softer substrates favour neurogenic outcomes and stiffer ones osteogenic, across laboratories and systems. Second, the mechanism is unresolved between bulk elasticity and adhesion-ligand presentation, and the two central papers in that dispute have never been reconciled. Third, elastic modulus is increasingly treated as a convenient proxy rather than the causal variable, with viscoelasticity and ligand presentation the live candidates.

The transcriptional consequences are also broader than lineage. RNA sequencing across substrate stiffness reveals diverse effects rather than a clean switch between three programmes [30], which suggests that reading stiffness as a lineage selector understates what it does.

This is a narrative review without systematic search or risk-of-bias assessment. Reported lineage windows are not comparable across studies because modulus was measured by different methods, and several quantitative values could not be confirmed at source and are therefore not reported.

Conclusion

Substrate stiffness influences mesenchymal lineage commitment, and the founding demonstration has proved

directionally reliable. It has not proved mechanistically settled: the tethering objection remains live, three-dimensional systems cannot separate stiffness from degradability or traction, stress relaxation reproduces osteogenesis at a modulus the two-dimensional literature assigns to muscle, and the same gel measures differently depending on how it is probed. Studies should report how the modulus was measured and at what indentation depth, state ligand density and anchoring chemistry, and where possible report stress relaxation alongside elasticity, so that the next set of windows can be compared with the last.

Table 1: Reported lineage windows, and the system each was measured in.			
System	Window	Outcome favoured	Ref.
2D polyacrylamide	0.1-1 kPa	Neurogenic	[5]
2D polyacrylamide	8-17 kPa	Myogenic	[5]
2D polyacrylamide	25-40 kPa	Osteogenic	[5]
3D alginate	11-30 kPa	Osteogenic	[18]
3D, fast stress relaxation	17 kPa	Osteogenic	[23]
2D polydimethylsiloxane	0.1 kPa - 2.3 MPa	No effect of stiffness	[15]
Same gels, method comparison	2-3 fold variation	Modulus depends on the probe	[28]

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Conflict of Interest

The authors declare that they have no competing interests relevant to the content of this review.

Ethics Statement

This article reviews previously published studies and contains no new studies with human participants or animals performed by any of the authors. Ethical approval was therefore not required.

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