

REVIEW

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# Extracellular matrix dynamics: tracking in biological systems and their implications

Michael Hu, Zihan Ling and Xi Ren\*

## Abstract

The extracellular matrix (ECM) constitutes the main acellular microenvironment of cells in almost all tissues and organs. The ECM not only provides mechanical support, but also mediates numerous biochemical interactions to guide cell survival, proliferation, differentiation, and migration. Thus, better understanding the everchanging temporal and spatial shifts in ECM composition and structure – the ECM dynamics – will provide fundamental insight regarding extracellular regulation of tissue homeostasis and how tissue states transition from one to another during diverse pathophysiological processes. This review outlines the mechanisms mediating ECM-cell interactions and highlights how changes in the ECM modulate tissue development and disease progression, using the lung as the primary model organ. We then discuss existing methodologies for revealing ECM compositional dynamics, with a particular focus on tracking newly synthesized ECM proteins. Finally, we discuss the ramifications ECM dynamics have on tissue engineering and how to implement spatial and temporal specific extracellular microenvironments into bioengineered tissues. Overall, this review communicates the current capabilities for studying native ECM dynamics and delineates new research directions in discovering and implementing ECM dynamics to push the frontier forward.

**Keywords:** Extracellular matrix (ECM), Lung, Proteomics, Newly synthesized protein, Stable isotope labeling by amino acids in cell culture (SILAC), Bioorthogonal non-canonical amino acid tagging (BONCAT), Bioprinting

## Introduction

The extracellular matrix (ECM) describes the noncellular network of macromolecules that is present in almost all biological tissues. Primarily composed of fibrous proteins and proteoglycans, the ECM functions to provide mechanical and biochemical support to cells within tissues, and helps drive key cellular events such as differentiation, migration, and proliferation [1]. The composition of ECM overall and how it varies in specific tissues and organs has been extensively reviewed [1–4]. However, few have focused on the changes in ECM composition over time: the transitional ECM differences between tissue states or between healthy homeostasis to pathological progression. These changes stem from the synthesis,

modification, and degradation of ECM biomolecules, in particular the protein components of the ECM, and are collectively referred to as ECM dynamics. ECM dynamics regulate cellular activities and affects tissue properties and functions; understanding these dynamic changes of the ECM is crucial to bettering our understanding of tissue pathophysiology and boosting our ability to engineer functional biological systems. In this review, we use the lung as the primary model organ to highlight what is changing in the ECM, its significance to the surrounding cells, how to measure these changes, and the implications these changes have for tissue engineering.

## The ECM and its dynamics

The ECM is generally composed of fibrous proteins for the major structural framework and heavily glycosylated proteoglycans for signaling and regulation purposes [1, 5–7]. Various proteins, such as collagens and elastin, are

\*Correspondence: xiren@cmu.edu

Department of Biomedical Engineering, Carnegie Mellon University, 5000 Forbes Avenue, Pittsburgh, PA 15213, USA



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synthesized as monomers in cells, post-translationally modified in the Golgi, packaged and released via secretory vesicles, and assembled into macromolecules in the extracellular space [8–13]. The resulting macromolecules commonly form fiber structures, such as elastic fibers - formed from elastin combining with glycoproteins like fibrillins and fibulins [14–16]- and fibrillar collagens like collagens I, II, III, V, and XI [17]. However, non-fibrous supramolecular structures are also assembled, as seen with the nonfibrillar collagen IV (lateral networks) and laminin (branched structures) [17–21]. These macromolecules are then crosslinked to one another by enzymes such as lysyl oxidase (LOX) to achieve desired mechanical properties and establish organ-specific tissue architectures [22–24]. For example, collagen IV and laminin are crosslinked to form the main architecture of the thin ECM surrounding cells, also known as basement membranes, while the fibrous proteins form 3D networks in the interstitial spaces [19, 20, 25–28]. These structural proteins coordinate with each other to deliver distinct mechanical characteristics under different stress levels. Taking the lung parenchyma as an example: at low stress levels, elastic fibers are the main load-bearing component, while at high stress levels the stiffer collagen fibers take over the load-bearing function, stiffening the tissue [4, 29, 30]. This 3D network also acts as a scaffold providing anchor points for other ECM proteins and cells to adhere to, allowing for transduction of extracellular mechanical forces such as stress and strain into signals for the cells to understand. Pulmonary models have demonstrated matrix stiffening mediating actin signaling to promote myofibroblast differentiation [31–33], and mesenchymal stem cells are known to change their morphology in response to the stiffness of the ECM that they are anchored to, transitioning their phenotypes from neuron-like, to myoblast-like, and to osteoblast-like with increasing matrix stiffness [34].

Proteoglycans, the other major component of the ECM, are each composed of a core protein with glycosaminoglycans (GAGs) attached and can be classified into multiple subgroups. Each subgroup matches the GAG type attached to the said proteoglycan: heparan sulfate (HS), chondroitin sulfate (CS), dermatan sulfate (DS), and keratan sulfate (KS) [35]. Proteoglycans can be held together through their interaction with another special type of GAG, hyaluronic acid (HA) [7, 36, 37]. As the largest biomolecule in the ECM, HA serves as a framework that non-covalently binds to the core proteins of proteoglycans, anchors these proteoglycans to the fibrous network, and together regulates ECM signaling [35, 38]. Furthermore, many proteoglycans have the inherent ability to bind growth factors in order to regulate their stability and diffusion within the ECM

and facilitate their interactions and signaling with cell surface receptors. For example, HS proteoglycans can facilitate fibroblast growth factor 2 (FGF2), hepatocyte growth factor (HGF), and transforming growth factor beta (TGF- $\beta$ ) signaling by immobilizing them via their heparin-binding domains [39–42]. Proteoglycan binding can also be inhibitory for the growth factor function. For example, decorin, a CS/DS proteoglycan, is reported to bind and inactivate TGF- $\beta$  to prevent fibril assembly and regulate cell differentiation during the pseudoglandular stage of lung development [35, 38, 43–45]. Proteoglycans play a significant role in tissue health; the removal of these proteoglycans from a decellularized lung ECM scaffold impairs the scaffold's ability to bind crucial ECM-associated growth factors, resulting in compromised ability to support cellular metabolism and growth [42].

While structural proteins and proteoglycans form the main structural foundation of the ECM, they only account for ~26% of ECM-associated genes; the rest of the matrisome – the global ECM and ECM-associated protein set – are associated with various regulatory functions [46, 47]. Regulatory proteins such as ECM-modifying enzymes (e.g., LOXs), cytokines, and growth factors, interact with and remodel the ECM through direct and indirect mechanisms. Together with the structural proteins and proteoglycans, these ECM-associated proteins interface with cells and the ECM itself to reshape the extracellular microenvironment as needed. ECM-cell signaling takes place through many biological mechanisms, such as cellular receptor signaling. To summarize, cell-surface receptors bind to the ECM and its associated biomolecules (growth factors, cytokines, etc.) to regulate key biological events such as cell adhesion, survival, and tissue morphogenesis [48, 49]. These receptors include membrane-embedded proteins such as integrins (activated by fibronectin, vitronectin, collagen, and laminin), growth factor receptors, discoidin domain receptors (activated by various types of collagens), and CD44 (receptor for HA) [50–54]. These ECM-cell interactions are crucial to healthy tissue development. For example, newborn lungs from mutant mice without the  $\alpha$ 3 integrin subunit displayed decreased branching from the major bronchi and altered epithelial morphology at the terminal respiratory branches from flattened to cuboidal [55]. Cell-surface receptors also engage in crosstalk with each other: integrins are well known for regulating growth factor receptors and vice versa [56]. Epidermal growth factor (EGF) receptors can be activated by integrins in absence of EGF ligand, and vascular endothelial growth factor (VEGF) can activate  $\alpha$ V $\beta$ 3,  $\alpha$ V $\beta$ 5,  $\alpha$ 5 $\beta$ 1, and  $\alpha$ 2 $\beta$ 1 integrins via the VEGFR2 receptor, affecting processes such as cell adhesion and migration [57, 58]

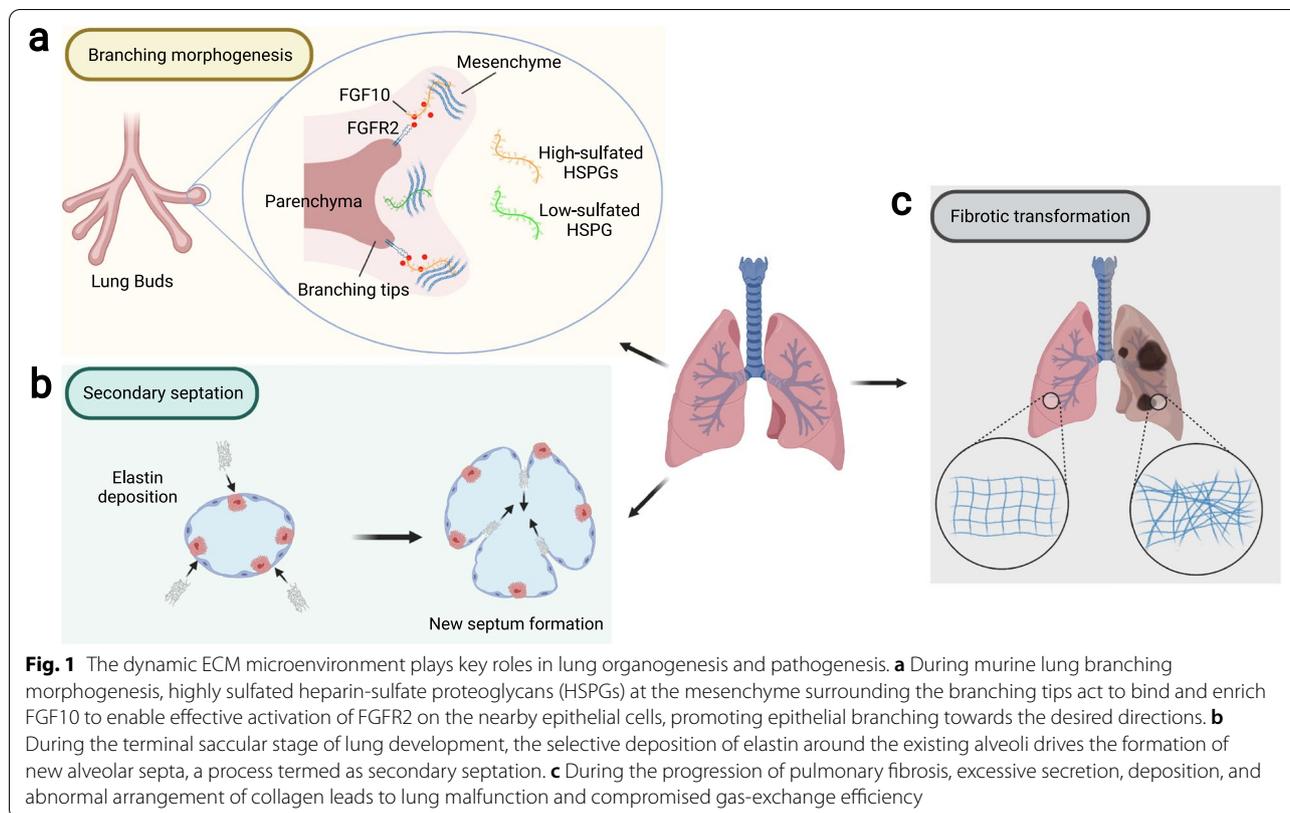
ECM-cell receptor signaling can be further regulated by proteolytic cleavage of matrix proteins during ECM remodeling, giving rise to peptide fragments, termed matricryptins, with newly revealed cryptic sites and bioactivities for cellular receptor interactions [49, 59–61]. Proline-glycine-proline (PGP), a tripeptide matricryptin derived from collagen I, is thought to be chemotactic to neutrophils and promote inflammatory responses in lung injury models that ultimately lead to fibrotic transformation [59, 62]. Mechanical force can also reveal cryptic sites; for example, fibronectin contains cryptic sites that can be exposed by tension. These sites are self-associative in nature and allow fibronectin to self-assemble at sites of high tension [63]. Aberrant myofibroblasts are thought to utilize this mechanism and pull on the ECM to stiffen tissues, resulting in fibrogenesis in organs such as the lungs [64].

All these ECM signals facilitate communication between different ECM components and between the ECM and cells to maintain proper tissue architecture and homeostasis. Spatially, the ECM can be divided into two categories: interstitial matrix and pericellular matrix [4, 7, 65]. Interstitial matrix makes up the bulk of ECM and contains most ECM components (collagens, fibronectins, proteoglycans) that are assembled into a GAG-rich matrix [1, 4, 66]. Pericellular matrix describes the ECM immediately surrounding cells, which possesses properties and compositions different from interstitial matrix and unique to the said cells; basement membranes can be considered a form of pericellular matrix unique to endothelial and epithelial cells and is composed of specific ECM components such as laminins, nidogens, perlecan, agrin, and collagen IV [7, 65, 67, 68]. In contrast, the pericellular matrix surrounding tendon cells is composed of collagen VI, versican, and fibrillin-2 [69]. Both interstitial and pericellular ECM are constantly being remodeled, and their properties are determined by the combined action of ECM modulators that keep each other in check. For example, the crosslinking LOXs and LOX-like enzymes are counterbalanced by the proteolytic activities mediated by matrix metalloproteinases (MMPs), which have an additional counterbalance with tissue inhibitors of metalloproteinases (TIMPs) [70–73]. These extracellular enzymes with opposing functions keep each other in balance and generate a matrix environment that is conducive to local cell activities.

However, sometimes it is desirable for there to be changes in matrix composition. These compositional ECM dynamics can be temporal or spatial in nature. Temporal differences in ECM composition occurs between different stages of biological processes such as wound healing and tissue development. For example, in lung injury models induced by allergen,

bleomycin, surgery, and naphthalene, matrix proteins taking part in the inflammation stage of lung wound healing (MMP2, TIMP1) are distinct from those involved in the subsequent re-epithelization stage (MMP7, MMP14) [74–79]. Similarly, during lung organogenesis, the ECM composition undergoes dynamic changes between each developmental stage to support temporal specific events in tissue morphogenesis and cellular specification [80, 81]. The murine fetal lung ECM consists of more proteoglycans and GAGs compared to that of the adult lung, which correlates with higher activity of ECM signaling for the rapid and massive changes in cellular phenotypes and tissue organization during embryonic lung development [81, 82]. Compared to the adult counterparts, fetal human and murine lungs express higher level of MMP-2 and lower level of TIMP-3, correlating with decreased ECM remodeling as the lung transitions from development to adult homeostasis [81, 83]. Further, elastin production is maximal during the terminal sacular stage of lung development, where elastin helps drive alveolar septation [81, 84].

Even within the same biological stage, there exists spatial differences in ECM composition across different organs, in the form of varying component ratios, organizations, and distributions of ECM components, thereby creating extracellular microenvironments with organotypic biological and mechanical properties. For example, elastic tissues like the lung and arteries are generally composed of collagen-and-elastin-rich matrix while the ECM of central neural systems are mostly GAG-rich structures [1, 6, 85–87]. Even within a particular organ, the composition varies spatially across different tissue compartments depending on their architecture and function. The interstitial matrix of the lung parenchyma is rich in type I and III collagen and elastin for contraction purposes while the basement membrane between the alveoli and nearby capillaries is rich in laminin, HA, and proteoglycans to support and facilitate gas exchange [4, 30, 87–89]. During development, spatial patterning of matrix proteins in the extracellular space helps guide cell migration and differentiation, leading to the establishment of heterogeneous organ structures. For example, the localization of FGF10 ligands to be adjacent to the branching tips of the nascent airway is critical for proper branching morphogenesis of the respiratory tracts, the process by which bronchi and bronchioles are formed [90]. It has been suggested that this FGF10 patterning is maintained by the heparin sulfates (HS) proteoglycans in the ECM, which store and concentrate FGF10 to be near the branching tips (Fig. 1a) [91, 92]. Similarly, during the process of secondary alveolar septation in lung alveologenesis, areas of high elastin content tend to form ring-like



structures on the alveolar epithelium that correspond to the tips of future septa (Fig. 1b) [93, 94].

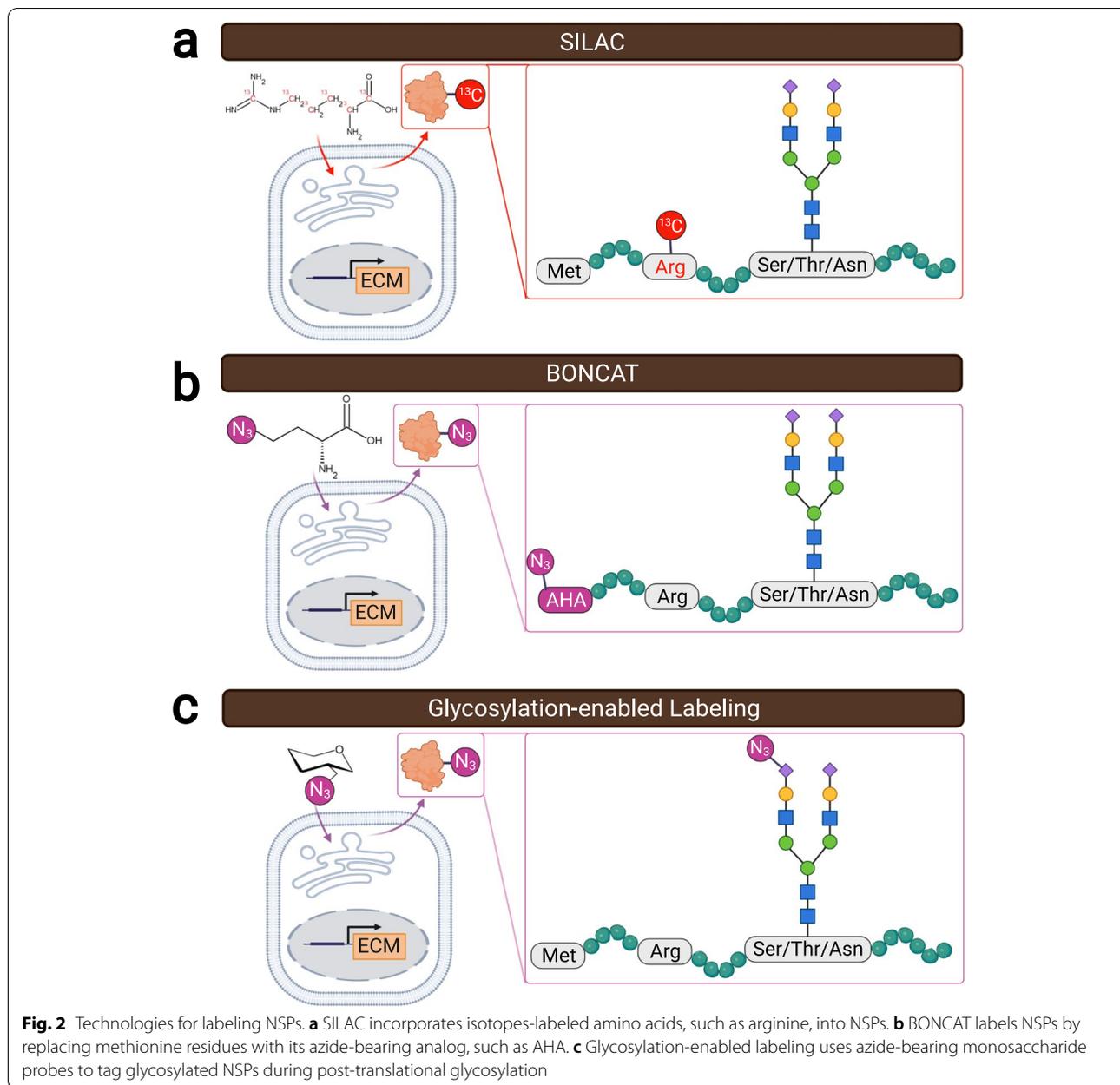
ECM alterations are usually well-controlled processes to achieve desired outcomes in tissue state transition, followed by reestablishment of homeostasis, but this is not always the case. Due to repetitive injury or aging, the ECM dynamics enter a positive feedback loop that can be detrimental to tissue health, leading to diseases such as fibrosis and cancer. Fibrosis has long been characterized as the excessive accumulation of collagens and other fibrous ECM components (Fig. 1c), while cancer disrupts normal ECM composition and creates its own, malignant extracellular environment [95, 96]. Furthermore, fibrotic ECM alone can induce profibrotic transformation of normal lung cells [97]. Existing tumors can release extracellular signaling molecules that look for and identify susceptible sites elsewhere in the body, and recruit tumor-associated cells (e.g., hematopoietic progenitor cells and macrophages) to remodel these sites' ECM to facilitate metastasis [98–100]. In both cases, the ECM becomes imbalanced and continues to spiral even further.

#### Tracking ECM dynamics

Tracking and understanding ECM dynamics provides fundamental insights regarding how diverse ECM

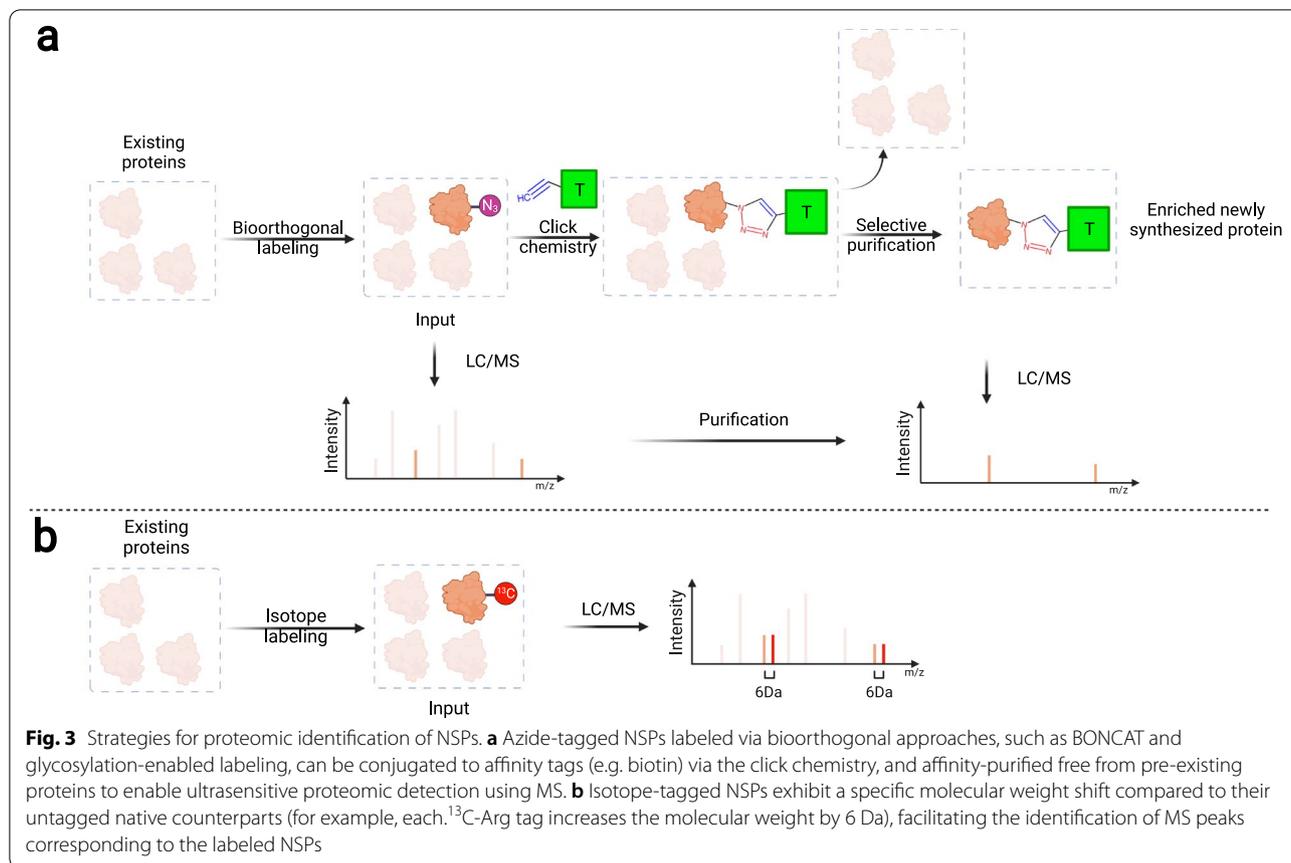
components function during tissue state transitions and help to reveal ECM signatures that correlate with tissue morphogenesis and pathogenesis. Mass spectrometry (MS) based proteomics is a popular approach to study the protein components of tissues, including the ECM. Conventional MS detects proteins with probabilities that are proportional to their abundances. While this is effective when investigating highly abundant protein species such as structural fibrous ECM components (e.g., collagens) or capturing dramatic changes in ECM composition accumulated over long time spans, MS proteomics falls short when the proteins of interest are in low abundance, having a transient expression profile, or both [3, 101]. Such challenges are commonly encountered when analyzing ECM dynamics as the newly produced ECM proteins are usually in extremely low abundance compared to the bulk pre-existing ECM, and sufficient compositional changes may take weeks of accumulation before they can be reliably detected [102, 103]. As a result, proteins in low abundance or with fast-changing kinetics have a high probability to be neglected by conventional proteomic analysis.

This gap in understanding dynamic ECM changes necessitates technology developments that allow detection and tracking of low-abundance, transient protein



species within the organotypic extracellular environment. Metabolic labeling of newly synthesized proteins (NSPs) is an attractive strategy as it allows incorporation of chemoselective tags into new protein additions, including ECM NSPs, during either protein translation or post-translational modification, allowing selective proteomic analysis of NSPs. Commonly used metabolic NSP labeling techniques include isotope labeling, bioorthogonal non-canonical amino acid tagging (BONCAT), and glycosylation-enabled NSP labeling (Fig. 2).

Isotope labeling of proteins can be accomplished via a variety of methods [101, 104], and a commonly used technique is stable isotope labeling by amino acids in cell culture (SILAC). Briefly, SILAC feeds and incorporates isotope-labeled amino acids, such as arginine, leucine, and lysine, into NSPs produced by cells (Fig. 2a) [105–107]. The resulting isotope-labeled NSP will display a signature mass shift compared to its non-labeled counterpart in MS analysis, and thus facilitate NSP detection (Fig. 3a). However, SILAC has been reported to require a long labeling period (at least 5 days) to label the murine



proteome *in vivo* and does not allow isolation of the isotope-labeled NSPs [108–110]. Nonetheless, recent efforts have validated the feasibility of using SILAC to quantify ECM protein deposition by cells repopulating decellularized human lung scaffold, where temporally specific matrisome changes were observed to correlate with cellular proliferative activity, cell adhesion, and ECM regeneration [111]. Similarly, in a tissue-engineered pulmonary fibrosis model, SILAC facilitated capturing differential expression of matrisomal proteins that underlined increased tissue density, stiffness, and ultimate force, as well as matrix-directed cellular responses in fibroblasts [112].

Bioorthogonal non-canonical amino acid tagging (BONCAT) operates by incorporating non-canonical amino acids (ncAAs) bearing chemoselective tags, such as azide-bearing methionine analog (L-azidohomocysteine, AHA) and alkyne-bearing methionine analog (L-homopropargylglycine, HPG), into the newly synthesized peptide chain during NSP translation (Fig. 2b) [113]. Some ncAAs, such as AHA and HPG, can be processed by endogenous aminoacyl tRNA synthetases (aaRSs) and utilized directly by cells for NSP production. Other ncAAs, such as azidonorleucine (ANL),

require mutant aaRSs to be attached to desired tRNAs and subsequently incorporated into NSPs. By controlling the tissue-specific expression of the needed mutant aaRSs, it is possible to track the spatial origin of NSPs using BONCAT, as only the proteins produced by the mutant-aaRS-expressing cells will be labeled [114–116]. The resulting azide/alkyne-labeled NSPs enable effective and selective bioconjugation to desired affinity probes bearing complementary tags via click chemistry, allowing for subsequent affinity purification for the labeled NSPs (Fig. 3b) [113, 117–119]. For this reason, BONCAT has exhibited impressive sensitivity and temporal resolution for NSP detection and has been reported to capture NSPs accumulated over as short as two hours [118]. In the last decade, BONCAT has mainly been used to study the dynamic synthesis of cellular proteins [114, 116, 120–122]. Only recently has BONCAT begun to be implemented for investigating the ECM, such as for visualizing ECM assembly and organization during chondrogenesis [123], for identifying NSP deposition by mesenchymal stem cells in hydrogels [124], and for revealing ECM compositional changes at different stages of mouse development [125, 126]. An emerging trend is to combine BONCAT and

SILAC to quantify proteins discovered via BONCAT. In this case, proteins are labeled with both ncAA and isotope labels; the ncAA labels allow for NSP enrichment and thus improved detection sensitivity, while the isotope labels allow for comparative quantification [93, 101, 109, 110].

Unlike SILAC and BONCAT, which directly label the amino acids, glycosylation-enabled labeling utilizes post-translational glycosylation to metabolically label the glycans attached to NSPs (Fig. 2c). Azide- or alkyne-bearing monosaccharide analogs of galactosamine and mannosaamine have been developed that specifically label glycosylated NSPs bearing O-linked mucin-type glycans or terminal sialic acid respectively [127–132]. Like BONCAT, once the azide or alkyne tags are incorporated into NSPs via their glycans, subsequent bioconjugation can be performed for visualization or purification purposes (Fig. 3b) [133, 134]. Since glycosylation occurs mainly to membrane-associated and secreted proteins and all ECM proteins are originally secreted by resident cells [135], it will be interesting to examine whether glycosylation-enabled NSP labeling has a labeling preference to matrisomal proteins as compared to SILAC or BONCAT. Future studies should focus on identifying, classifying, and quantifying which proteins are tagged via glycosylation-enabled NSP labeling. Our recent study has validated the use of azido galactosamine analog to label the ECM-NSPs in a wide variety of tissues and organs (such as the lung, heart, liver, kidney, skin, and blood vessel) *in vivo* as well as during *ex vivo* lung culture [136]. This *ex vivo* labeling ability opens up the opportunity to label donor human tissues and thus perform more clinically relevant studies. Further focus on selective analysis of matrisomal NSPs can be achieved when combining proteomic analysis with selective extraction of ECM proteins via decellularization to remove most cellular components. Moreover, glycosylation-enabled labeling can be and has been used to study glycan composition in ECM, *i.e.*, ECM glycomics [37, 137–142].

These above-described metabolic labeling methods can be combined with MS to identify changes in ECM protein synthesis. While this review primarily focuses on ECM compositional dynamics, it is important to note that these methods are not limited to only studying compositional changes. BONCAT and glycosylation-enabled labeling allows fluorophore conjugation to ECM-NSPs to visualize and measure the new additions of ECM architecture in tissues [123, 124]. Furthermore, protein–protein interactions and protein topography (*i.e.*, mapping a protein's internal structure), topics that are crucial to understanding ECM assembly and architecture, can be studied by modifying BONCAT and using mutant tRNA to insert ncAAs onto pre-determined sites on a protein.

The inserted ncAAs can then help identify or alter parts of the protein structure and its interaction with other proteins [114, 115, 143, 144]. We direct the readers to the following reviews for more information on site-specific ncAA labeling and its applications [114, 115, 143].

#### Implications of ECM dynamics for ECM engineering

Investigation of ECM dynamics hold implications for biomaterial and tissue engineering by increasing our understanding of native, organotypic ECM compositions, processes, and interactions. Protein patterns discovered through ECM dynamics studies can be recreated in tissue scaffolds to better support or guide cells. These changes may be spatial, temporal, or even spatiotemporal in nature and would require different technologies to capture the nuances of each change. Here, we briefly go over the current states and limits of emerging technologies that would be useful for implementing these ECM changes in engineered tissue morphogenesis.

Spatial ECM patterns, differences, and architecture can be recreated via 3D bioprinting techniques. 3D bioprinting is biofabrication method that allows for the 3D arrangement of cells and biomaterials in a layer-by-layer fashion and has been noted for replicating complex tissue geometries with high precision, such as microfibril muscle structures, entangled vascular networks with oxygen exchange abilities, and neonatal scale whole-heart scaffolds [145–154]. However, these bioprints are generally homogenous in material and printed with compositionally simple bioinks, such as collagen and poly(ethylene glycol) diacrylate (PEGDA), which remain distant from fully recapitulating the complexity of the ECM composition found in native organs. This has not been unnoticed by the field and recent advances in technology development are beginning to allow 3D bioprinting of multiple bioinks, enabling the embedment of multi-material patterns in prints and bringing the resulting constructs closer to replicating spatial ECM heterogeneity [155–158]. Decellularized ECM, from a variety of sources such as the heart, blood vessel, and airway mucosal tissues, has been used recently to generate bioinks with improved tissue-specificity [159, 160]. However, the decellularization process often relies on harsh detergents and has been shown to strip important GAGs and their associated growth factors from the ECM of organs such as the lungs [42]; this gap will need to be addressed to enable better recapitulation of native ECM composition using decellularized ECM bioinks. Investigation regarding ECM dynamics during native tissue morphogenesis holds the potential to discover new important components within the extracellular space, providing critical instruction for formulating more physiologically relevant bioinks for tissue engineering [4, 159].

Apart from bioink formulation, bioprinting also face spatial patterning limitations. Much of native ECM 3D structure remains not completely understood, and without better understanding ECM structure, we are, in a sense, missing blueprints to print from. Common ECM components such as collagens and laminins have had their structures mapped out via techniques such as second harmonic imaging and immunostaining [161–164], but many other ECM protein patterns remain elusive to us. Recent investigations are beginning to bridge these knowledge gaps: decellularization has been used to isolate ECM components with preserved, organ-specific composition and architecture [159, 162–164], various sensor array strategies have been developed for MS and RNA sequencing to discretely divide tissue samples into analyzable “voxels” and spatially track ECM proteomics and transcriptomics [165–167], and nascent matrices have been labeled to study their assembly and growth in hydrogels [123, 168]. However, ECM composition and structure are complex, and it is still unclear which proteins and what structures in the decellularized ECM bioinks result in the reported improved cellular support [159]. Further attention should be directed towards better understanding these proteins and their matrix architectures. Additionally, out of the ECM patterns that we do know, some are too small to be printed; pulmonary capillaries are  $\sim 7 \mu\text{m}$  in diameter and basement membranes are 50–100 nm thick, whereas most bioprinting techniques have resolutions in the tens of micrometers [169–171].

ECM composition is not only spatially specific, but also temporally specific. Data from ECM dynamics studies could be used to generate an ECM composition that is specific to a particular stage of organ development or pathological progression, allowing the engineered biomaterials to model temporal snapshots of the tissue of interest. Stage-specific ECM signaling molecules can be introduced to tissue scaffolds via adjustments to the scaffold production chemistries or chemical linkage to existing scaffolds. For example, ECM scaffolds have been functionalized with growth factors such as TGF- $\beta$ 1 and FGF2 to better drive cell differentiation and support cell proliferation respectively [172, 173]. Unfortunately, these scaffolds and scaffolds created by most other ECM biofabrication techniques face a common limitation in that they only capture the ECM composition of one particular stage instead of the evolving ECM microenvironment as is commonly seen in native biological processes. To address this bottleneck, there has been interest in scaffold-incorporable, activable functional groups that can be engineered to respond to outside variables and change the scaffold's properties, effectively allowing temporal control and even some spatial control over said scaffold

properties. This has manifested in scaffolds responsive to multiple stimuli, with photostimulation being the most heavily investigated for tissue engineering purposes [174]. Light has been used to control biochemical cue presentation and mechanical properties in scaffolds through various photochemical strategies such as photocaging, radical polymerization, and crosslink degradation. Photocaging involves adding photosensitive groups to mask the bioactivities of the target biomolecule. After these masked biomolecules are incorporated into scaffolds, light can be shined on them to cleave off the photosensitive group and restore biomolecule functionality [175]. This has been utilized to spatiotemporally control growth factor localization and cell migration [176]. Radical polymerization involves chemistry that allows UV light to generate more radicals for polymerization, increasing the density and stiffness of the scaffold in locations exposed to UV light. A common example of this is methacrylate-based polymerization, which is currently popular in tissue engineering and has been utilized to guide cell differentiation within scaffolds [177]. In contrast, scaffolds can also be made to be photodegradable by making crucial scaffold elements such as crosslinkers photodegradable; this has been utilized to spatiotemporally degrade locations in scaffolds to give room for organoids to develop into and form structures [178, 179]. Apart from light, scaffolds responsive to other stimuli such as ultrasound, magnetic fields, and electric signals have been developed and are actively researched. We recommend the following review for a more in depth look at photo-responsive, ultrasound-responsive, magnetically responsive, and electrically responsive tissue scaffolds [174].

### Concluding remarks

The ECM is a crucial component of all tissues not only as a structural support, but also as a signaling system that communicates environmental cues to cells and influence a wide variety of cellular activities. As tissues change due to development, growth, or disease, the ECM is also being modified to both drive and accommodate said changes. Effective ECM engineering is thus critical for recapitulating the dynamic, evolving extracellular microenvironment necessary for engineered tissue morphogenesis. However, such efforts are hindered by our limited understanding of the ECM dynamics accompanying native biological processes. Much protein profiling has been done to the matrisome, but many current discovery strategies are unable to detect low-abundance, transient protein species. To combat this, new strategies are focusing on the ECM fraction that undergoes active changes such as new ECM synthesis. SILAC has been used to study

ECM-NSPs, but its application is limited by its inability to isolate the NSPs from the pre-existing protein background. This challenge is being addressed by the BONCAT technology, which is just beginning to focus on ECM-NSPs, and we look forward to the discoveries that will follow. Glycosylation-enabled labeling has been in literature used to study new ECM accumulation and has recently been demonstrated to work in multiple organs in vivo and ex vivo; efforts to incorporate proteomics with glycosylation-enabled labeling should bear new findings for ECM dynamics. Additionally, just as SILAC and BONCAT have been combined to create a methodology that allows for BONCAT's purification and SILAC's quantification steps, glycosylation-enabled labeling could be combine with SILAC to deliver robust quantitative assessment of glycosylated NSPs that is highly prevalent in the matrisome. These research directions will uncover previously unknown ECM proteins that are associated with key developmental or pathological events and map out such event-specific ECM microenvironments spatially and temporally. Apart from protein composition, ECM dynamics studies focused on glycomics, protein–protein interaction, and protein topography would shed further light on additional ECM properties such as 3D structure and biochemical gradients. Extracellular vesicles embedded in ECM have recently been tied to ECM-cell interactions [180–184], and Tenascin-R, a key brain ECM protein, has been observed being endocytosed and resurfacing later, essentially being “recycled” during matrix remodeling [185]. These discoveries bring in additional layers of complexity in understanding how the evolving extracellular microenvironment mediates pathophysiological processes. Knowledge gained from studying these phenomena can then be acted upon via engineered strategies such as 3D bioprinting and ECM functionalization, creating biomimetic ECM conditions to guide cell behaviors and functions.

#### Abbreviations

ECM: Extracellular matrix; LOX: Lysyl oxidase; HS: Heparin sulfate; CS: Chondroitin sulfate; DS: Dermatan sulfate; KS: Keratan sulfate; HA: Hyaluronic acid; FGF2: Fibroblast growth factor 2; HGF: Hepatocyte growth factor; TGF- $\beta$ : Transforming growth factor  $\beta$ ; EGF: Epidermal growth factor; VEGF: Vascular epithelial growth factor; PGP: Proline-glycyl-proline; MMP: Matrix metalloproteinase; TIMP: Tissue inhibitors of metalloproteinase; HSPG: Heparin sulfate proteoglycans; MS: Mass spectrometry; NSP: Newly synthesized protein; BONCAT: Bioorthogonal non-canonical amino acid tagging; SILAC: Stable isotope labelling by amino acids in cell culture; ncAA: Non-canonical amino acid; AHA: L-azidohomoalanine; HPG: L-homopropargylglycine; tRNA: Transfer RNA; aaRS: Aminoacyl tRNA synthetase; ANL: Azidonorleucine; PEGDA: Poly(ethylene glycol) diacrylate.

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The schematics were created using BioRender.

#### Authors' contributions

M.H. and X.R. designed the structure of the manuscript. M.H. contributed to the written sections to the manuscript. Z.L. contributed to the figures and figure legends. All authors read and approved the final manuscript.

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#### Declarations

##### Ethics approval and consent to participate

N/A.

##### Consent for publication

N/A.

##### Competing interests

The authors declare that they have no competing interest.

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