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The provisional matrix: setting the stage for tissue repair outcomes

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Abstract

Since its conceptualization in the 1980s, the provisional matrix has often been characterized as a simple fibrin-containing scaffold for wound healing that supports the nascent blood clot and is functionally distinct from the basement membrane. However subsequent advances have shown that this matrix is far from passive, with distinct compositional differences as the wound matures, and providing an active role for wound remodeling. Here we review the stages of this matrix, provide an update on the state of our understanding of provisional matrix, and present some of the outstanding issues related to the provisional matrix, its components, and their assembly and use *in vivo*.

Introduction

The provisional extracellular matrix (ECM), or provisional matrix, is a term first coined in the early 1980s by R.A. Clark when describing factors that appeared coincident with epidermal cell migration during skin wound healing [1]. In this seminal paper, Clark et al. describes the ECM present in biopsies of skin during wound healing and finds that a fibrin- and fibronectin-rich thicken ECM emerges early in the repair process following injury. Whereas laminin and collagen IV, two components of the epidermal basement membrane, promote "tenacious epithelial attachment" and limit epithelial migration, the fibrin- and fibronectin-rich ECM appeared to stimulate migration. At the conclusion of wound closure, the fibrin and fibronectin had disappeared and the normalcy of the basement membrane returned. While Clark stopped short of declaring the necessity of a fibrin- and fibronectin-rich ECM in epidermal wound repair, he did help to define a new type of ECM and launch what has become an area of intense investigation into the role ECM plays in guiding tissue repair and explicitly in the "provisional matrix". For the purposes of this Special Issue, the "Provisional Matrix" is defined similarly as R.A. Clark originally defined it, a proteoglycan-containing fibrin- and fibronectin-rich ECM that emerges immediately following injury, as a consequence of blood coagulation in response to vascular damage, that enables tissue repair. Thus the provisional matrix, as its name implies, is an acute, temporary ECM and is quickly degraded and replaced by cells during wound repair.

Subsequently the definition of provisional matrix was refined in the 1990s by Magnus Magnusson and Dean Mosher who split the provisional matrix phase of wound repair into "early" and "late" phases [2]. There is indeed a critical distinction between the composition, structure, and function of the provisional matrix that forms immediately following vascular injury (the early provisional matrix) and a resident cell-derived provisional matrix that emerges within the early phases of repair. As shown schematically in Fig. 1 and described below and throughout this issue, this matrix changes with time to affect different cells as they return following injury.

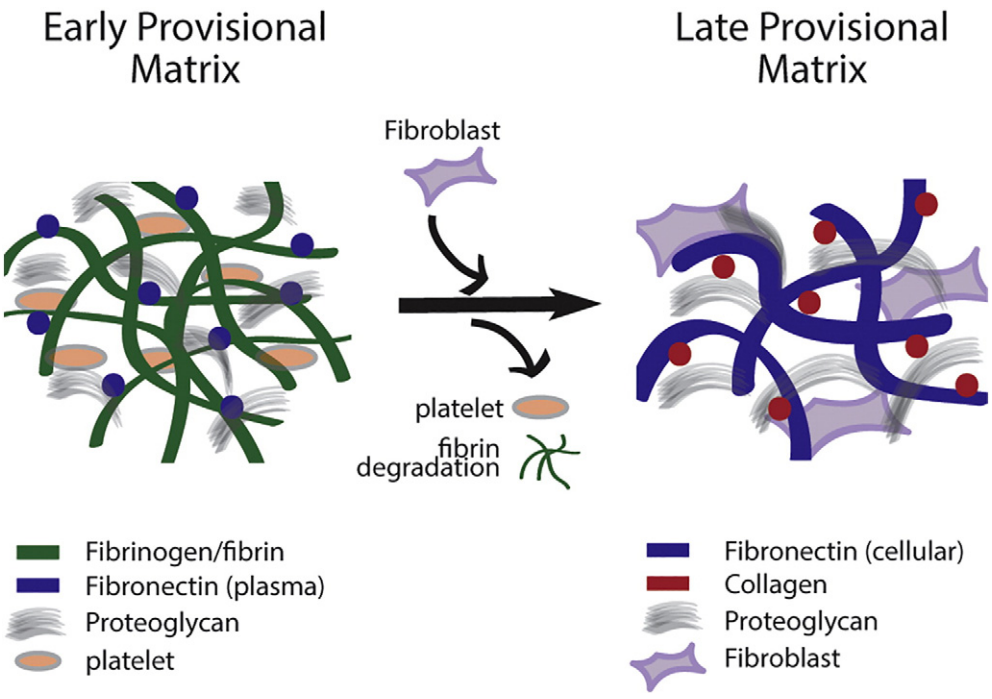

Fig. 1. Schematic of the formation and progression of Provisional Matrix over time. The early provisional matrix is formed in response to vascular injury triggering of the clotting cascade. The early provisional matrix is a fibrin rich polymer with interspersed crosslinked plasma fibronectin. The contents of platelet α-granules are released into the fibrin-rich matrix and contribute to the complexity of this earliest matrix. As the matrix then matures, cell-mediated remodeling (directed primarily by inflammatory cells and fibroblasts) takes place and includes a transition from a fibrin- to fibronectin-dominated scaffold containing a significant proteoglycan component and interspersed crosslinked collagen.

Early Provisional Matrix

This early matrix is dominated by the clotting response to vascular permeability and is primarily comprised of acute phase serum proteins and proteins from within the α-granules of platelets, which are activated during clot formation. As a consequence, the early provisional matrix is a fibrin-rich polymer with interspersed, crosslinked plasma fibronectin. The primary function of this early provisional matrix is to provide a polymer that can assemble into a mechanical stabile network. Such a network can then entrap platelet plugs to stem blood loss and provide temporary scaffolding for subsequent host cell migration and invasion. Despite decades of research on fibrinogen, fibrin and clots, there remain a plethora of unanswered questions related to the intricacies of fibrin formation, the biophysical properties of fibrin, and polymer structure and how variants of fibrinogen impact clot dynamics and cardiovascular disease. For example although the structure/assembly of fibrin monomers within a fibrin protofibril are “regular”, meaning there is a regular half-staggered structure to fibrin protofibrils based on the molecular positions of polymerization knobs and their complimentary binding holes [3], there remain several critical questions. These include what is the flexibility of surrounding fibers, what is the stability of protein-protein interactions during polymerization and their impact on structure, and what is the αC domain of the molecule, which plays numerous and varied roles in polymerization dynamics, cell-association, and clot dissolution?

Work within this special issue first provides a historical perspective on fibrinogen and fibrin research by Russ Doolittle (in this issue). Subsequent authors attempt to clarify the issues above, especially as they relate to how protein splicing (Duval and Ariens and Cronjé et al., in this issue) and composition impacts matrix formation and physical properties (Litvinov and Weisel, Chester and Brown, and Kim et al. in this issue).

Late Provisional Matrix

As the provisional matrix matures into later phases, the early provisional fibrin-rich matrix, is replaced primarily by fibronectin and proteoglycans. Fibronectin plays fundamentally critical roles in wound repair from regulating cell adhesion dynamics to providing the essential ECM template for subsequent collagen deposition [4]. Unlike fibrin, whose polymerization is triggered and regulated by limited and specific interactions between monomers, fibronectin assembly is driven through a complicated

process of cell-binding, molecular extension of the protein through physical forces to expose multiple cryptic self-association sites [5]. In the polymerized state, fibronectin can be viewed as a scaffold signaling protein. Again work within this issue highlight down stream signals initiated by fibronectin assembly, including integrin binding and force production.

Fibronectin's critical role in guiding tissue morphogenesis and repair stem from (A) its multiple binding sites for both the early provisional matrix protein fibrin and the permanent matrix protein collagen, (B) its promiscuous integrin binding domains which are juxtapositioned to a growth factor and proteoglycan binding domain, and (C) its molecular extensibility. Indeed, fibronectin has been shown to be a critical link to permanent matrix deposition and in its absence collagen fibers are insufficiently stabilized prior to secondary crosslinking [4]. Fibronectin also displays significant molecular deformation within the matrix, a function attributed to the extensibility of its type III repeats [6]. These biophysical alterations of fibronectin's structure potentially lead to an additional level of physiological control of its function, which is reviewed by Zollinger and Smith (in this issue). The multitude of possible binding interactions and their position relative to one another enables the potential of dynamic allosteric control impacting availability of fibronectin-binding growth factors, synergistic signaling, e.g. syndecans, etc., and controling cell integrin binding. Several manuscripts in this special issue directly extend this concept of dynamic allosteric control over the provisional matrix; for example, maturing provisional matrix requires the co-assembly of hyaluronic acid and versican for its stability (Wight, in this issue) and is regulated by stromal cells, which link themselves to this matrix both via syndecan-4-mediated extracellular attachments and via cell-cell adhesion (Gopal et al, in this issue). Within the context of these cell-ECM adhesions, Chen et al (in this issue) extent the concept of assembly regulation to the integrin itself, noting the significant tension-induced conformational changes of αVβ3 integrin in real-time are required for activation.

Provisional Matrix and Signaling

Separately, the provisional matrix acts as a significant source of growth factors, i.e. fibronectin contains many cryptic binding sites for growth factors, such as TGFβ. Additional force is also required to liberate these factors from their fibronectin-rich matrices [7] to impact cell behavior. Griggs et al. (in this issue) examines this concept by illustrating how mature fibronectin fibrils regulate TGFβ binding. As cells contract against this matrix, they further show that the matrix can act as a TGFβ source to induce Epithelial-Mesenchymal Transition (EMT) and cancer. Beyond force, fibronectin-heparin interactions alter the structure of the growth factor binding domains on fibronectin and may impact which growth factors bind during various stages of wound repair [8] whereas co-clustering of cell surface integrin and heparin-sulfate proteoglycans, like syndecans, have been shown to induce differential signal transduction pathways [9]. It is clear that although we appreciate the potential biological implications of fibronectin dynamics, there remain critical unanswered questions about its role in orchestrating late stage matrix and cell binding whose answers are essential to our understanding of the provisional matrix's role in tissue repair.

Interactions Beyond the Matrix

Components of the provisional matrix are not limited to wound healing, and it is important to note their ubiquitous expression, interactions with other ECM, and use in other tissues. While there are obvious wound healing-relevant pathologies like fibrosis, Harris et al. (in this issue) highlight the interactions between fibronectin deposited by stromal cells and neurons, which use it as a growth cue. Patterning such matrices could use provisional matrix components to our advantage to direct their growth in therapeutic applications. Conversely, some cells directly support stromal cell assembly as occurs in malignant cancers. Unlike with EMT, malignant breast cancer cells can alter stromal cell assembly dynamics by reorganizing fibronectin matrix themselves as they migrate through collagen matrices (Wang et al, in this issue). Interactions need not be direct as in these examples as indirect signaling and regulation can occur from delivery of matrix cargo by extracellular vesicles. As Song et al. describe (in this issue), breast cancer-derived vesicles can stimulate adipose stem cells to alter their association with ECM and differentiate down a myofibroblast lineage. From these contributions, it is clear that provisional matrix components cannot be thought of only in the context of wound healing, but that their indirect roles in a variety of other systems is critical and often based on their function within the provisional matrix itself, e.g. assembly changes and signaling within the clot.

Thus, in this Special Issue of *Matrix Biology*, we have assembled an excellent group of authors, all experts in fibrinogen, fibrin, and fibronectin and many of whom are historical leaders in the field. In step with the natural progression of the provisional matrix, from early to late, we have organized the Issue by first focusing on fibrinogen and fibrin followed by fibronectin, then moving into applications of provisional matrix in wound repair and pathology.

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References

[1] R.A. Clark, J.M. Lanigan, P. DellaPelle, E. Manseau, H.F. Dvorak, R.B. Colvin, Fibronectin and fibrin provide a provisional matrix for epidermal cell migration during wound reepithelialization, J. Invest. Dermatol. 79 (5) (1982) 264–269.

[2] M.K. Magnusson, D.F. Mosher, Fibronectin: structure, assembly, and cardiovascular implications, Arterioscler. Thromb. Vasc. Biol. 18 (9) (1998) 1363–1370.

[3] G. Tsurupa, A. Mahid, Y. Veklich, J.W. Weisel, L. Medved, Structure, stability, and interaction of fibrin alphaC-domain polymers, Biochemistry 50 (37) (2011) 8028–8037.

[4] J. Sottile, D.C. Hocking, Fibronectin polymerization regulates the composition and stability of extracellular matrix fibrils and cell-matrix adhesions, Mol. Biol. Cell 13 (10) (2002) 3546–3559.

[5] P. Singh, C. Carraher, J.E. Schwarzbauer, Assembly of fibronectin extracellular matrix, Annu. Rev. Cell Dev. Biol. 26 (2010) 397–419.

[6] M.L. Smith, D. Gourdon, W.C. Little, K.E. Kubow, R.A. Eguiluz, S. Luna-Morris, V. Vogel, Force-induced unfolding of fibronectin in the extracellular matrix of living cells, PLoS Biol. 5 (10) (2007), e268. .

[7] B. Hinz, The extracellular matrix and transforming growth factor-beta1: Tale of a strained relationship, Matrix Biol. 47 (2015) 54–65.

[8] B. Hubbard, J.A. Buczek-Thomas, M.A. Nugent, M.L. Smith, Heparin-dependent regulation of fibronectin matrix conformation, Matrix Biol. 34 (2014) 124–131.

[9] A. Woods, J.R. Couchman, Syndecans: synergistic activators of cell adhesion, Trends Cell Biol. 8 (5) (1998) 189–192.