

Author's Accepted Manuscript

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PII: S0012-1606(17)30543-2
DOI: <https://doi.org/10.1016/j.ydbio.2017.12.022>
Reference: YDBIO7660

To appear in: *Developmental Biology*

Received date: 10 August 2017
Revised date: 16 December 2017
Accepted date: 30 December 2017

Cite this article as: Andrea Acuna, Michael A. Drakopoulos, Yue Leng, Craig J. Goergen and Sarah Calve, Three-dimensional visualization of extracellular matrix networks during murine development, *Developmental Biology*, <https://doi.org/10.1016/j.ydbio.2017.12.022>

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Three-dimensional visualization of extracellular matrix networks during murine development

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Abstract

The extracellular matrix (ECM) plays a crucial role in embryogenesis, serving both as a substrate to which cells attach and as an active regulator of cell behavior. However, little is known about the spatiotemporal expression patterns and 3D structure of ECM proteins during embryonic development. The lack of suitable methods to visualize the embryonic ECM is largely responsible for this gap, posing a major technical challenge for biologists and tissue engineers. Here, we describe a method of viewing the 3D organization of the ECM using a polyacrylamide-based hydrogel to provide a 3D framework within developing murine embryos. After removal of soluble proteins using sodium dodecyl sulfate, confocal microscopy was used to visualize the 3D distribution of independent ECM networks in multiple developing tissues, including the forelimb, eye, and spinal cord. Comparative analysis of E12.5 and E14.5 autopods revealed proteoglycan-rich fibrils maintain connections between the epidermis and the underlying tendon and cartilage, indicating a role for the ECM during musculoskeletal assembly and demonstrating that our method can be a powerful tool for defining the spatiotemporal distribution of the ECM during embryogenesis.

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