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Modular GAG-matrices to promote mammary epithelial morphogenesis *in vitro*

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ABSTRACT

Matrix systems used to study complex three-dimensional (3D) cellular processes like mammary epithelial tissue morphogenesis and tumorigenesis *ex vivo* often require ill-defined biological components, which lead to poor reproducibility and a lack of control over physical parameters. In this study, a well-defined, tunable synthetic biohybrid hydrogel composed of the glycosaminoglycan heparin, star-shaped poly(ethylene glycol) (starPEG), and matrix metalloproteinase- (MMP-) cleavable crosslinkers was applied to dissect the biophysical and biochemical signals promoting human mammary epithelial cell (MEC) morphogenesis. We show that compliant starPEG-heparin matrices promote the development of polarized MEC acini. Both the presence of heparin and MMP-cleavable crosslinks are essential in facilitating MEC morphogenesis without supplementation of exogenous adhesion ligands. In this system, MECs secrete and organize laminin in basement membrane-like assemblies to promote integrin signaling and drive acinar development. Therefore, starPEG-heparin hydrogels provide a versatile platform to study mammary epithelial tissue morphogenesis in a chemically defined and precisely tunable 3D *in vitro* microenvironment. The system allows investigation of biophysical and biochemical aspects of mammary gland biology and potentially a variety of other organoid culture studies.

1. Introduction

Mammary gland development and homeostasis are tightly regulated and depend on diverse instructive signals from a highly complex biochemical and biophysical extracellular microenvironment. In the last two decades, three-dimensional (3D) *in vitro* culture models of mammary morphogenesis have successfully contributed to deciphering the mechanisms underlying crucial aspects of mammary epithelial development and tumorigenesis (reviewed in Refs. [1–4]). However, most 3D *in vitro* mammary epithelial studies have been conducted using biologically-derived extracellular matrix (ECM) biomaterials, such as a basement membrane extract (BME, commercially known as Matrigel®) or hydrogels which self-assemble from the fibrillar ECM protein collagen I. In addition to their self-assembly into soft, 3D scaffolds, both of these matrices either contain or include exogenously supplemented ligands such as laminin (LN) to recapitulate aspects of the native basement membrane of epithelial cells. Non-transformed mammary epithelial cells (MECs, e.g. MCF10A cell line) embedded as single cells in these biologically-derived matrices proliferate and develop into growth-arrested, polarized multi-cellular spherical organoids, which closely resemble the terminal ductal lobular units (acini) of the native human mammary epithelium (Fig. 1A) [2,5–8]. Because of this remarkable recapitulation of native morphogenesis, BME has been the “gold standard” culture platform for modeling *ex vivo* mammary epithelial development.

Despite their utility, naturally-derived biomaterials have several significant intrinsic limitations, most notably a xenogeneic origin and a complex, ill-defined composition [9]. Furthermore, natural biomaterials lack methodological flexibility. For example, it is

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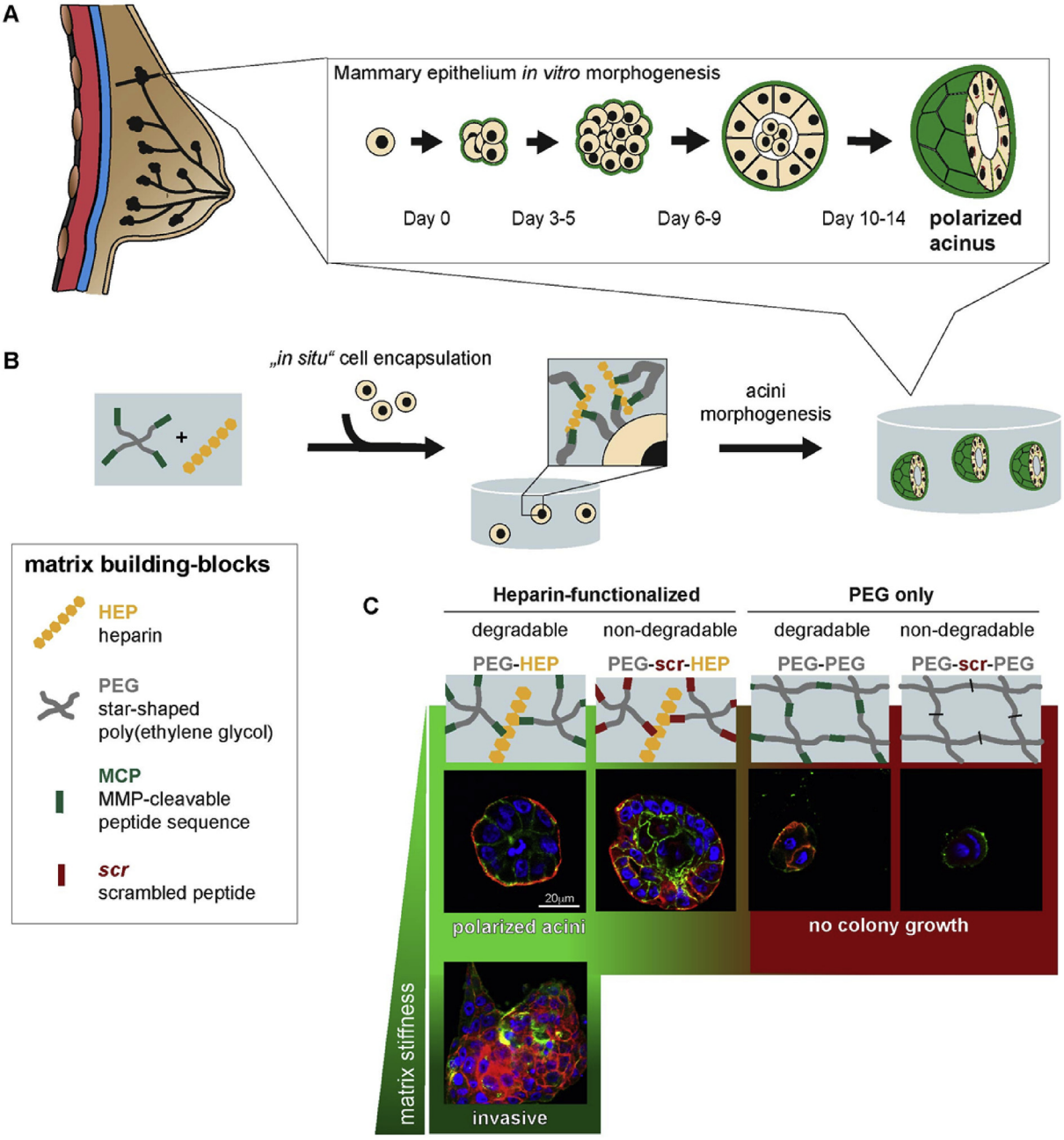

Fig. 1. Mammary epithelial cell morphogenesis *ex vivo* in a modular biomimetic hydrogel. (A) Diagram of *in vitro* individual mammary epithelial cell (MEC) morphogenesis into polarized acinus displaying a cleared lumen and basement membrane formation (green). **(B)** Illustration of the study design to investigate MEC morphogenesis in a hydrogel matrix which contains the following building blocks: glycosaminoglycan (GAG) heparin (yellow), MMP-cleavable peptide sequence (MCP, green), non-cleavable scrambled peptide sequence (scr, red) and the inert and star-shaped, four-armed PEG (grey) as a structural matrix component. The versatile and modular chemistry of the biomimetic matrix building blocks facilitates the study of the specific function of the different matrix building blocks and the exogenous biophysical (mechanical) stimuli on mammary epithelial morphogenesis in a systematic fashion. MECs are embedded as single cells with the modular matrix prior to polymerization. Over 14 days in culture, they undergo morphogenesis to form polarized mammary epithelial acini. (**C**) Polarized MEC acini formation after 14 days in culture is optimal in soft, enzymatically degradable PEG-HEP hydrogels. In each of the other combinations of modular building blocks (e.g. starPEG, heparin, MMP-cleavability) or matrix stiffness, MEC morphogenesis is perturbed. Scale bar = 20 µm.

difficult to independently modulate their mechanical and biochemical properties, which limits their utility for measuring the impact of these cell-instructive microenvironment cues on cellular development and homeostasis. These limitations have profound impacts on cell response, experimental outcome, reproducibility, and comparative studies (reviewed in Refs. [3,9]).

In response to these limitations, numerous synthetic biomaterials have been successfully engineered for 3D cell culture and tissue engineering applications [10–12]. Two commonly used synthetic biomaterials are poly(ethylene glycol)-(PEG) based hydrogels functionalized with specific cell-adhesion and degradation motifs and materials which self-assemble from peptides into fiber-like matrices. Only a few of these materials have been specifically designed or adapted to study 3D MEC morphogenesis [13–15], and these attempts have often lacked the design flexibility to independently examine various matrix properties. Thus, well-defined 3D synthetic matrices that allow the systematic investigation of the distinct contributions of biochemical and mechanical