# Chapter I. Introduction

Cellular organization into specialized, functional multicellular structures is achieved through dynamic interactions between cells and their surrounding microenvironment (1). The microenvironment presents instructions for orchestrating many cellular processes, including proliferation, migration, and differentiation in a spatio-temporally coordinated manner (2). Tight regulation of these cell behaviors in a multicellular context is essential for organ development, function, and homeostasis. Meanwhile, perturbations among environmental cues and/or in the cellular apparatus that senses and responds to these cues leads to significant pathological consequences, such as cancer development (3).

Epithelial tissues exhibit highly-ordered cell-cell junctions and polarized structures, mainly serving a barrier function for protection, partitioning, and sensation (4). In addition to its structural role, cell-cell contact is a key factor regulating epithelial tissue growth. Contact-inhibition of proliferation is a hallmark of normal epithelial cells, and the loss of contact-inhibition results in chaotic proliferation, leading to tumor formation (5). Given its role in cancer, “contact-inhibition” has been the subject of extensive research ever since it was first described in the early 1960s at a phenomenological level in a culture that had reached saturation density (6). Contact-inhibition is now better understood with greater resolution at the molecular and cellular levels.

While progress continues in uncovering the physicochemical mechanisms mediating contact-inhibition (7-12), the quantitative aspects of this key constraint are unclear. In particular, contact-inhibition and the loss of this constraint occur in a complex microenvironment replete with conflicting cues such as soluble growth factors (GF) and extracellular matrix (ECM). GFs bind receptors on the cell surface and activate a set of downstream intracellular signaling pathways that can stimulate proliferation (13). Cells are also anchored to the surrounding ECM whose physical and chemical properties regulate cellular mechanics (14-15) and adhesion-dependent growth signaling (16-17).

It remains unclear how contact-inhibition is enforced in such a complex microenvironment that includes multiple, potentially conflicting, cues. What perturbations in these environments potentially lead to the loss of contact-inhibition, transitioning the system to a contact-independent state? Ultimately, how do cells quantitatively integrate and converge these differential inputs into a net decision on cell cycle? Addressing these questions will provide insight into a pivotal step in the self-organization of multicellular systems during development and the disruption of multicellular morphology during cancer progression.

Deciphering quantitative principles of contact-inhibition can also offer design strategies for biomedical applications, such as tissue engineering and regenerative medicine. A quantitative understanding of contact-inhibition of proliferation is needed

to manipulate multicellular growth patterns and rates in synthetic microenvironments. Recent advances in material design (18-19) and microfabrication (20-21) techniques have enabled *in situ* fine-tuning of the degree of and context in which cells form contacts with their neighbors and the matrix. Furthermore, spatio-temporally controlled release of soluble growth factors is feasible with the use of advanced polymeric materials (22), microfluidics (23-24), and MEMS devices (25). How these powerful technologies to manipulate environmental cues may be applied to tune rationally the growth and organization of multicellular structures is a key engineering challenge.

Using a quantitative approach at a single cell level within two-dimensional multicellular aggregates, we elucidate a quantitative framework for contact-inhibition of proliferation when cells are presented with conflicting cues – cell-cell contact (growth-inhibitory) and EGF (growth-promoting) (Chapter 2). Our results demonstrate that epithelial cells transition between contact-inhibited and contact-independent modes of proliferation when the amount of EGF crosses a critical threshold level. Only when the level of EGF recedes to this threshold level, do contacts effectively suppress cell cycle activity among interior cells, driving a spatially patterned, contact-inhibited growth state. Furthermore, this transition point is tunable. We show that augmenting cell-cell contacts using micropatterned surfaces and molecular approaches enables contact-inhibition at a higher EGF threshold.

This state diagram perspective of contact-inhibition suggests that the attenuation

of contact-inhibition may occur progressively over the course of oncogenesis as cancer-promoting perturbations gradually accumulate in the epithelial system and surrounding environments. We directly tested this hypothesis by measuring the quantitative effects of stiffening the adhesive matrix, a broadly observed phenomenon during *in vivo* tumorigenesis (Chapter 3). We show that even when substratum stiffening has no apparent effect on contact-inhibition at a phenotypic level, it markedly reduces the threshold amount of EGF, quantitatively shifting normal cells closer to the transition line to contact-independence. By using the proximity to this transition point as a metric, we demonstrate that quantitative changes in matrix compliance modulate the "degree" of contact-inhibition. These potent effects of matrix stiffening involve the erosion of contact-maturation, which alters the subcellular localization of EGF receptor as well as cell-cell adhesion molecules. Moreover, we demonstrate that substratum compliance and EGF synergistically modulate multicellular mechanics in three-dimensions, which correspond to multicellular growth patterns (Chapter 4).

In summary, we elucidate quantitative principles for contact-inhibition co-regulated by cell-cell contact, EGF, and substratum compliance with implications in modulating the degree of contact-inhibition and multicellular growth patterns. The proposed quantitative model of contact-inhibition enhances our understanding of cancer progression and offers design principles for engineering spatial patterns and rates of growth of multicellular structures.

**References**


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