·专题报告·

组织工程中的生物力学问题

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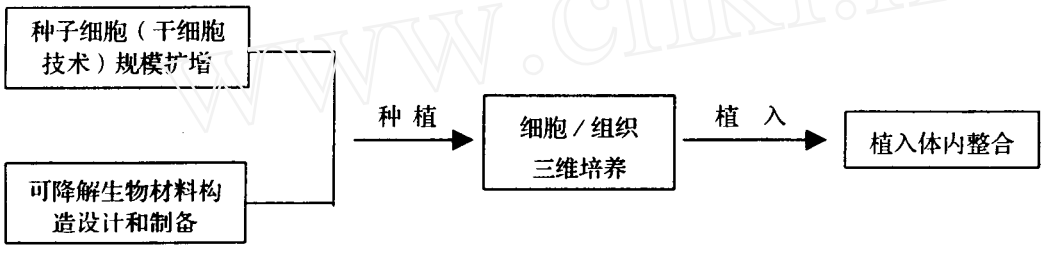
组织工程(Tissue Engineering)是近十年来兴起的一个新的前沿领域。1987年在美国NSF发起的一次会议上提出了"Tissue Engineering"一词，1988年，NSF的一个专门工作小组对组织工程的内涵做了如下界定："应用工程科学和生命科学的原理和方法来解释正常的和病理的哺乳动物的组织和器官的结构－功能关系，并且发展具有生物活性的人工代替物，来恢复、维持或提高组织、器官的功能"。据此，组织工程的科学内涵有三个紧密结合的部分：

1.对哺乳动物组织、器官(正常的和病理的)结构－功能关系(定性→定量)的认识；

2.在可控(可重复)条件下，通过哺乳动物特定细胞的体外培养，形成具有活性的生物替代物，包括具有特定功能的组织、细胞－骨架聚集体、细胞悬浮液、细胞及其产物的包囊、生物人工器官(bioartifical organ)等。

3.离体培养组织(或生物替代物)植入后和机体组织的相互作用和整合。

如果说(2)是组织工程的主体，那么，(1)是它的基础，(3)则是它的应用、检验和效益的体现。就(2)而言，其程式可简式如下：



这里，核心问题是分化，即诱导种子细胞定向分化，长成具有特定功能的组织或器官。

种子细胞具有可分化的多能性，而种子细胞的规模扩增，要求在大规模自我更新(增殖)过程中，不失去分化的多能性，这不仅仅决定于细胞本身和生长因子(生化微环境)，而且与培养过程中种子细胞的力学环境(应力、应变、流型等)有密切关系。

可降解生物材料及构架制备是组织工程的另一个重要基础，与一般医用生物材料相比，除了生物相容性(血液相容性、免疫抗性等等)外，有其特殊性，主要是：(1)维持诱导、细胞定向分化；(2)控制降解速率，使得 $t_D/t_G \sim \theta(1)$，t_D：由于材料降解构架强度衰减特征时间；t_G：组织生长强度提高特征时间；(3)降解产物无毒；(4)构架不仅是提供细胞生长的基底，而是要利于传质(营养和氧的供应，代谢产物的排除)。

(2),(4)显然和生物力学有关。而(1)和生物相容性的本质在于：

- 细胞——材料表面相互作用；
- 生物大分子——材料表面相互作用。

90年代的研究表明，mechano-chemical effects 在此起着重要作用，这是走向21世纪的生物力学的一个新生长点。

细胞/组织三维培养是组织工程之所以称为工程的关键性的一步，也是形成组织工程产业的必要的科学技术基础。这里，培养系统(bioreactor)内的流动与传质规律，应力－细胞生长、分化关系，以及细胞/组织培养过程中力学环境(微环境)的调控起着关键性作用。

不仅仅如此，无论是在体发育还是离体细胞/组织培养，整个过程都和细胞的聚集、黏附、变形、运动并不断地改变其生物学图式(biological pattern)位移，都是其基本运动形式，而力学正是研究以位移为特征的机械运动的科学，这些都是生物力学的研究对象。

综上可见，无论是广义而言，还是狭义地讲；无论从科学基础层面，还是从工程技术层面来看，生物力学都是组织工程的不可或缺的基础。大体而言，主要包括：

1. 组织、器官在体力学环境(应力分布等)的分析和在体力学环境外模拟(调控)；
2. 应力—细胞发育、增殖、分化的关系；

3. 细胞—材料表面之间的 mechano-chemical effects；
4. 细胞黏附、变形、运动的力学规律及生物学图式形成和变化的动力学规律；
5. 离体培养组织植入体内后和机体组织的相互作用；
6. 细胞/ 组织三维培养系统的流动和传质规律；
7. 离体培养组织的力学性能及其和自然组织性能的比较。等等，等等。

组织工程是 21 世纪生物医学工程的突出的前沿。它的发展将大大推动 21 世纪医学的进步，同时也为 21 世纪生物力学的发展开辟了一个新天地。这里，至为重要的一点是：力学和生命科学的真正结合。对于有志于此的力学工作者来说,应当深深铭记：只有失去自我（已有的）才能在新领域里重新塑造自我。

THE KINETICS AND MECHANICS OF CELL ADHESION MOLECULES

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ABSTRACT

A unifying theory for the molecular biomechanics of receptor-ligand interaction is being developed, which is based on the probabilistic formulation for kinetics of small systems plus the constitutive laws describing the dependence of kinetic rates on force. A diverse set of experiments were performed to test the theory, including those using various cells and molecules, being conducted under both steady and transient conditions, and employing different techniques including centrifugation, micropipette, and rosetting. Specific solutions to each experiment were obtained, many of which are analytical. The satisfactory comparisons between predictions and data not only validate the theory but also enable determination of the constitutive equations and the associated parameters.

INTRODUCTION

Binding via adhesive receptors is essential to many cellular functions. The kinetic rates are critical to such interactions since these parameters determine how rapidly cells bind and how long they remain bound. In contrast to soluble molecules, the association and dissociation of bonds cross-bridging two apposing surfaces usually take place in the presence of forces, as one of the biological functions of the adhesion molecules is to provide mechanical linkage between cells. Physical forces can influence the binding interactions of adhesive bonds. As such, the chemical kinetics of receptor-ligand binding is tightly coupled to the mechanics of stretching and breaking these bonds at the molecular level. Also, the number of bonds formed is usually small owing to small number of interacting molecules and/or low affinity. Consequently, the bond number may fluctuate significantly as the result of the stochastic nature of the individual molecular kinetics.

THEORY

To account for random fluctuations in the kinetics of small systems, the probabilistic formulation (master equations) of McQuarrie was adapted:

$$\frac{dp_n}{dt} = m_r m_l A_c k_f\left(\frac{f}{n}\right)p_{n-1} - [m_r m_l A_c k_f\left(\frac{f}{n+1}\right) + nk_r\left(\frac{f}{n}\right)]p_n + (n+1)k_r\left(\frac{f}{n+1}\right)p_{n+1}$$

where p_n is the probability of having n bonds at time t, m_r and m_l are the respective densities of receptors and ligands in the contact area A_c. k_f and k_r are, respectively, the forward and reverse rate coefficients, the dependence of which on applied force f are described by the constitutive equations:

$$k_r(f) = k_r^0 \exp[\alpha(\frac{af}{k_B T}) + \beta(\frac{af}{k_B T})^2]$$

$$K_a(f) \equiv k_f(f)/k_r(f) = K_a^0 \exp[-(\frac{af}{k_B T})^b]/[1 + c(\frac{af}{k_B T})^d]$$

where k_B is the Boltzmann constant and T the absolute temperature. k_r^0 (in s^{-1}), K_a^0 (in μm^2), a (in nm), and α, β, b-d (dimensionless) are parameters.

RESULTS

The steady-state solution of the master equations has been applied to the centrifugation experiment to measure 2D affinity. A representative result is exemplified in Fig. 1.

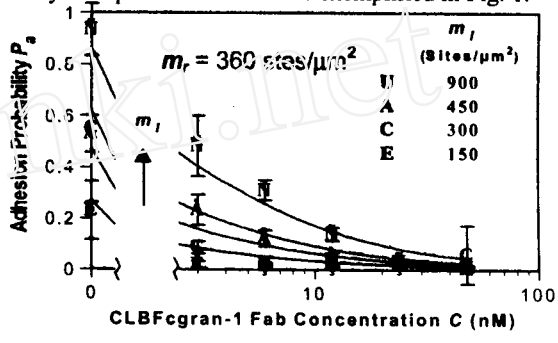

Fig. 1. Binding of CD16a-expressing CHO cells to surface-bound RbIgG in the presence of anti-CD16 mAb assayed by centrifugation. Data (points) are presented as mean ± SD of quadruplicate wells. The curves are a single least chi-squares fit of theory to all data.

The zero-force transient solution has been applied to the micropipette experiment to measure 2D kinetic rates. A representative result is exemplified in Fig. 2.

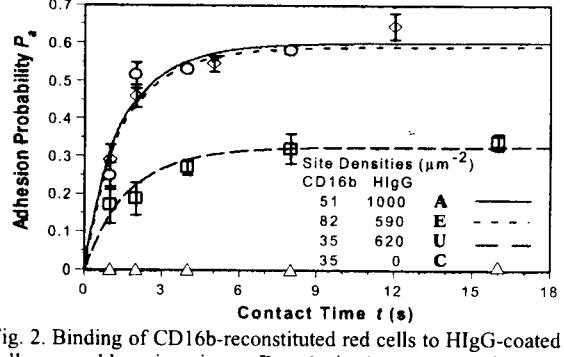

Fig. 2. Binding of CD16b-reconstituted red cells to HIgG-coated red cells assayed by micropipette. Data (points) are presented as mean ± SE of 1-8 pairs of cells with 100 contact each. The curves are a single least chi-squares fit of theory to all data.

The ability of the theory to account for data of different experiments and to derive the same values for the intrinsic parameters when the same receptor/ligand systems are used have provided strong support for our unifying theory. Using molecules genetically-engineered to alter their structures and cells transfected to express these molecules in various forms, the relationships among the structure, property and function of biological cells and molecules can be defined, which are the goals for this work.

ACKNOWLEDGEMENTS

Supported by NSF grant BCS-9350370 and NIH grant R29 AI38282.