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Commentary

Weaving cartilage at zero *g*: the reality of tissue engineering in space

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Proem

tissue *n* [L, *texere*, to weave] . . . an aggregate of cells usu. of a particular kind together with their intercellular substance . . . (1)

On Arachne, the weaver:
Oft, to admire the niceness of her skill,
The Nymphs would quit their fountain, shade, or hill:
. . .
Nor would the work, when finish'd please so much,
As, while she wrought, to view each graceful touch (2)

Tissue Exchange

The idea of tissue exchange between individuals is captivating. Fantasized hybrid beings, with parts from various sources, acquire unusual stature and reflect an ageless anxiety; the Sphinx, Chimera, and Frankenstein's creation linger in our imaginations. Real hybrids also engage us, as when, 30 years ago, in the first human-to-human heart transplant, the diseased heart of Louis Washkansky was replaced with the heart of a young female accident victim (3). Since that dramatic operation, the images of hybridization have become ever more complex; technology reforms our mythology. A motion picture released in 1997 asked viewers to accept the possibility of two individuals exchanging faces; it is a measure of progress that this remarkable conceit could be presented credibly.

The new field of tissue engineering asks for a similar suspension of disbelief. Tissue engineers are attempting to invent recipes—lifted from developmental biology, transplant surgery, chemical engineering and other disparate sources—that will cause biological tissues to regenerate *in situ* or develop *in vitro* (4). The field is propelled by progress in gene therapy, cell biology, polymer science, and controlled drug delivery (5–7). If recent trends continue, laboratory-grown tissue replacements will become a common medical therapy during the early decades of the 21st century, just 100 years after the first culture of animal cells (8). Cell culture is a marvelous innovation of modern biology: cells rapidly reproduce their own structure if provided essential nutrients and maintained in a balanced, sterile environment. During histogenesis, however, another level of structure must be produced; individual cells are enjoined to coordinate and specialize by mechanisms that are not yet understood. What are the determinants of cell organization and differentiation in tissues? Can development be manipulated to permit the fine control of tissue architecture that is necessary for "synthetic" tissues to become useful clinical products?

In this issue of the *Proceedings*, Freed *et al*. (9) introduce a new concept in tissue engineering: control of the gravitational field during histogenesis as a means of modulating tissue structure and function. The technology involved in these experiments is staggering: chondrocytes were harvested from adult tissue, seeded upon customized polymer templates, cultured in specialized bioreactors on Earth for 3 months, carried into orbit by the Space Shuttle, transferred to the Mir space station for an additional 4 months of culture, and returned to Earth for analysis. The experimental design alone gives one reason to pause, but the result is equally compelling: cartilage development was noticeably different on Earth and in space, suggesting that some feature of the microgravity environment influenced subsequent tissue development.

Tissue Engineering

Tissue exchange is an ancient art, of which tissue engineering is the latest genre. Blood transfusion was described by ancient Egyptian and Old Testament writers (10). Folklore refers to transplantation of the nose (frequently lost by sword or syphilis); skin and cartilage replacements were performed by Sushruta as early as 1000 B.C. and Tagliacozzi during the Renaissance (11). Skin grafts to other sites were common by the 1800s; Churchill donated skin to a comrade in 1898 (12). Although human heart transplantation was not accomplished until 1967, the concept existed with Huo T'o in third century China (11). The "modern" age of tissue exchange began in the 1940s, but transplantation did not become widespread until immunosuppressive drugs were discovered. Today, success of tissue transplantation has stringent limits, which are determined primarily by the availability of donor tissue. Many people with end-stage tissue failure, but with good prospects for survival after transplantation, die waiting for donor tissue. Tissue engineering developed from the urge to create larger supplies of transplantable tissue, perhaps produced from a patient's healthy cells or engineered cell lines.

How can tissue development in culture be manipulated? Some of the mechanisms of cell organization during tissue development are known (Fig. 1 *A–C*). Laboratory techniques for inducing tissue formation, frequently inspired by natural mechanisms, are available in certain cases (Fig. 1 *D–F*). Central among these new strategies is the idea, first proposed less than 10 years ago (13), that synthetic materials can serve as degradable templates for tissue regeneration (Fig. 1*E*). Blood cells circulate, so they can be transplanted by injection and still find their proper place by native homing mechanisms. Cells of other tissues are localized at a particular site; transplanted cells want a mechanical foundation and chances for cell–cell communication. With biodegradable polymer scaffolds, the overall tissue size and shape can be molded; cells can be provided surfaces for anchorage; microgeometry can be optimized for cell recruitment; and the synthetic polymer can be programmed to dissolve as the tissue form emerges. It is now well known that viability and function of surface-attached cells depend on properties of the surface (ref. 14; reviewed in ref. 15). In fact, synthetic surfaces can be chemically modified to replicate the chemical (6) and physical (16, 17) features of tissues, rendering materials active for certain cell populations.

In the new work by Freed *et al.* (9), a poly(glycolic acid) mesh supports a cell population; as described in previous reports, these woven scaffolds provide an environment in which cells proliferate, function, secrete proteins, and organize into a tissue that resembles natural cartilage in important respects. By taking identical constructs, organized during 3 months of

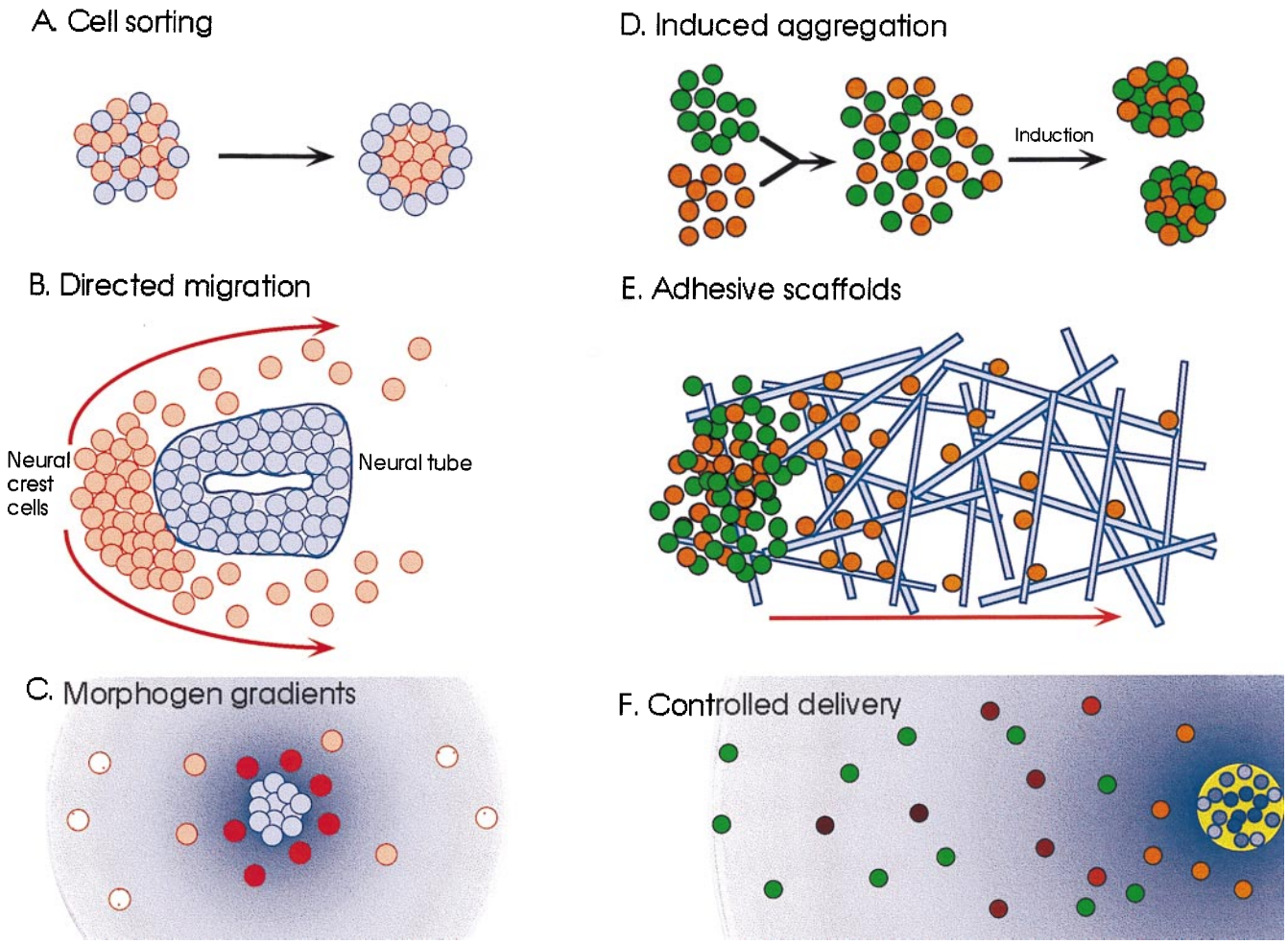

FIG. 1. During histogenesis, cells (indicated by red and blue spheres) assemble into a functional tissue. Cell assembly and differentiation occur by a number of mechanisms during embryogenesis, including cell sorting into layers (20), which is guided by cell–cell adhesion molecules (*A*); directed migration along adhesive pathways (21), which requires interactions with extracellular matrix, cell surfaces, and diffusible substances (*B*); and differentiation in gradients of morphogens (22), which provide positional information in the developing tissue (23) (*C*). In *B*, cells from the neural crest migrate around the neural tube and aggregate to form ganglia at distal tissue sites. In *C*, the red cells acquire positional information by the local concentration of a factor released by the blue cells. In tissue engineering, these natural processes are mimicked by using cells (indicated by orange and green spheres) and synthetic approaches such as induction of aggregation, which can occur by addition of polymers that stimulate cell adhesion (24) (*D*); provision of scaffolds (4), which control microarchitecture and adhesion for invasion by specific cells (*E*); and sustained release of bioactive agents from localized sources (25) (*F*). In *D*, assembly of a suspension of cultured cells, mixed to form the correct proportions, is induced by additional of a soluble cell–cell adhesion promoter; in *E*, the matrix of polymer fibers supports the adhesion and migration of the orange cells, but not the green; and in *F*, the gradient of morphogen, which gives positional information only to the orange cells, is provided by a controlled release implant. (*B* was adapted from ref. 26, figures 19–21.)

initial culture on Earth, and continuing culture either on Earth and in space, Freed tested the hypothesis that further tissue development would depend on gravity. Indeed, the space-grown constructs were more spherical, contained less glycosaminoglycan (GAG), and were mechanically inferior to constructs grown on Earth. These changes were observed in constructs with similar numbers of functional cells, as judged by proline and sulfate incorporation rates. The data are consistent with the idea that tissue function (i.e., mechanical strength) depends on structure (i.e., GAG content), which results from cell activity. Here, production of functional GAG was enhanced on Earth, presumably because of differences in mechanical forces, which are known to influence chondrocyte function. Mechanical forces, presented to cells by fluid shear (18) or direct manipulation of the surface (19), can influence cell function at multiple levels.

Will tissues of the future be grown in space? The present study provides good evidence that *in vitro* tissue development in space is different than that on Earth, but the study does not yet provide a definitive mechanism to exploit. The scarcity, inadequacy, and high cost of donor tissue provide sufficient justification for heroic new efforts, however, and herein resides the power of this new work. The microgravity environment suggests new ways to isolate mechanisms in the evolution of tissue structure. Here, for the first time, macroscopic pieces of tissue were maintained in suspension, external forces stripped to those necessary to mix chemicals in the vessel. Motions on the smallest scale—diffusion, convection, cell migration, and receptor adhesion—must be balanced and weighed if we are to interpret the promenade of molecules and cells engaged in tissue formation. In this respect, the low gravity environment of space offers an unprecedented opportunity for studying complex fluid assemblies, such as the one studied here.

Tissue Weaving

For Arachne, her weaving was simply too good: she offended the goddess Athena with her audacity as well as her skill. As punishment, Arachne was transformed into a spider, forever weaving, forever captive of the web. Like the pioneers of tissue

exchange, we must be confident yet circumspect in our application of new science. An exquisite synthetic tissue could create more problems than a rude one, particularly for strongly "human" tissues such as the brain and the heart. The article by Freed *et al*. will stimulate speculation, and probably controversy. But one thing is undeniable: tissue engineering has left the surface of the Earth and is now free from the forces that usually regulate tissue development, and our analysis of tissue development. If tissue engineering is to be successful, it will require this kind of thinking and working beyond the normal limits.

1. (1984) *Webster's Ninth New Collegiate Dictionary* (Merriam-Webster Inc., Springfield, MA).
2. Ovid in Metamorphoses, VI, lines 19–20, 27–28 (translated into English verse under the direction of Sir S. Garth by J. Dryden, A. Pope, J. Addison, W. Congreve, *et al.*).
3. Barnard, C. N. & Pepper, C. B. (1969) *One Life* (George G. Harrap & Co. Ltd., London).
4. Langer, R. & Vacanti, J. P. (1993) *Science* **260,** 920–932.
5. Baldwin, S. P. & Saltzman, W. M. (1996) *Trends Polymer Sci.* **4,** 177–182.
6. Hubbell, J. A. (1995) *Bio/Technology* **13,** 565–576.
7. Koller, M. R. & Palsson, B. O. (1993) *Biotechnol. Bioeng.* **42,** 909–930.
8. Harrison, R. G. (1907) *Proc. Soc. Exp. Biol. Med.* **4,** 140–143.
9. Freed, L. E., Langer, R., Martin, I., Pellis, N. R. & Vunjak-Novakovic, G. (1997) *Proc. Natl. Acad. Sci. USA* **94,** 13885–13890.
10. Oberman, H. (1981) in *Clinical Practice of Blood Transfusion*, eds. Petz, L. D. & Swisher, S. N. (Churchill Livingstone, New York), p. 9.
11. Deaton, J. G. (1974) *New Parts for Old: The Age of Organ Transplants* (Franklin Publishing Company, Inc., Palisade, NJ).
12. Churchill, W. L. S. (1930) *My Early Life, a Roving Commission* (Butterworth, London).
13. Vacanti, J. P., Morse, M., Domb, A., Saltzman, W. M., Perez-Atayde, A. & Langer, R. (1988) *J. Pediatr. Surg.* **23,** 3–9.
14. Folkman, J. & Moscona, A. (1978) *Nature (London)* **273,** 345–349.
15. Saltzman, W. M. (1996) in *Cell Interactions with Polymers*, eds. Lanza, R., Chick, W. L. & Langer, R. (R. G. Landes, Co., New York), pp. 227–248.
16. Singhvi, R., Kumar, A., Lopez, G. P., Stephanopoulos, G. N., Wang, I. C., Whitesides, G. M. & Ingber, D. E. (1994) *Science* **264,** 696–698.
17. Clark, P., Connolly, P., Curtis, A. S. G., Dow, J. A. T. & Wilkinson, C. D. W. (1991) *J. Cell Sci.* **99,** 73–77.
18. McIntire, L. V. (1994) *Ann. Biomed. Eng.* **22,** 2–13.
19. Wang, N., Butler, J. P. & Ingber, D. E. (1993) *Science* **260,** 1124–1127.
20. Steinberg, M. (1963) *Science* **141,** 401–408.
21. Bronner-Fraser, M. (1993) *J. Neurobiol.* **24,** 133–247.
22. Driever, W. & Nusslein-Volhard, C. (1988) *Cell* **54,** 83–93.
23. Wolpert, L. (1971) *Curr. Topics Dev. Biol.* **6,** 183–224.
24. Dai, W., Belt, J. & Saltzman, W. M. (1994) *Bio/Technology* **12,** 797–801.
25. Saltzman, W. M. (1996) *MRS Bull.* **21,** 62–65.
26. Alberts, B., Bray, D., Lewis, J., Raff, M., Roberts, K. & Watson, J. D. (1994) *Molecular Biology of the Cell* (Garland , New York).