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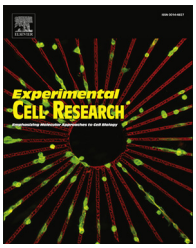

Review Article

In the beginning there were soft collagen-cell gels: towards better 3D connective tissue models?

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ABSTRACT

In the 40 years since Elsdale and Bard's analysis of fibroblast culture in collagen gels we have moved far beyond the concept that such 3D fibril network systems are better models than monolayer cultures. This review analyses key aspects of that progression of models, against a background of what exactly each model system tries to mimic. This story tracks our increasing understanding of fibroblast responses to soft collagen gels, in particularly their cytoskeletal contraction, migration and integrin attachment. The focus on fibroblast mechano-function has generated models designed to directly measure the overall force generated by fibroblast populations, their reaction to external loads and the role of the matrix structure. Key steps along this evolution of 3D collagen models have been designed to mimic normal skin, wound repair, tissue morphogenesis and remodelling, growth and contracture during scarring/fibrosis. As new models are developed to understand cell-mechanical function in connective tissues the *collagen material* has become progressively more important, now being engineered to mimic more complex aspects of native extracellular matrix structure. These have included collagen fibril density, alignment and hierarchical structure, controlling material stiffness and anisotropy. But of these, tissue-like collagen density is key in that it contributes to control of the others. It is concluded that across this 40 year window major progress has been made towards establishing a *family* of 3D experimental collagen tissue-models, suitable to investigate normal and pathological fibroblast mechano-functions.

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In the beginning: soft, random and homogeneous

The origins of the use of soft, cell-seeded collagen gels goes back many decades (how many depends on the author). It is perhaps an opportune moment, though, to mark the 40th anniversary of the pioneering paper of Elsdale and Bard [1]. Their contribution was significant, not least for its striking opening prose and its clear description of cell-spatial mechanics as demonstrated by fibroblasts, shuffling through soft 3D collagen gels.

The Elsdale and Bard opening is famous for its picturesque personification of cells. But in fact it also stresses the 3D collagen 'lattice' as the fibroblast dwelling-place, its living SPACE. "*Snatched from a life of obscurity and installed in contemporary glass and plastic palaces, cells are in danger of becoming Pygmalion's protégés. Housed in more traditional residences constructed of water and collagen instead of plastic or glass, do cells lead primitive, less cultured lives*?" Although the pun on "cultured" is priceless, it is more likely that 'Space' is the key-word here, making it essential to consider the solid and fluid components, the mechanical properties of that material, and how those mechanical properties change with time, scale, and orientation. They pondered the key factors which should probably still engage us more than they do:

i. the native collagen fibrillar structure (as a 'lattice');
ii. the difference in spatial cues for cells grown IN or ON the gel, and
iii. the ability to control structural alignment, by physical cues (including flow shear during gelling).

Interestingly enough these authors made very little of the later focus on how such 3D cell–gel constructs change shape (or contract) as cells attach to and generate their own forces onto the collagen fibrillar net. They did, however, discuss the difference of cell shape and attachment in collagen versus agar gels. This led them to stress the differences in cell response where attachment was minimal (agar), or at one surface to an ultra-stiff material (plastic) or over all their surfaces to a compliant collagen. Certainly, in tackling the question of how stromal fibroblasts deposit, grow, remodel and repair their matrix, a 3D collagen-based model was a huge leap forwards relative to culture on stiff plastic, as a naked cell monolayer. By analogy this might be compared to experiencing a balloon flight in the balloon basket (ie. 3D collagen) as opposed to watching it on a flat screen TV.

However, Elsdale and Bard also made passing mention to a detail which then took almost 4 decades to attract much attention again. The density of solid support material (collagen fibrils) in such soft, hyper-hydrated gels is trivial. The starting concentration of collagen is 0.1%, by weight (more recently rising only to 0.5%), meaning that such gels can be as much as 99.9% water! If we are honest, this is the real magic of 'the collagen gel model'. Indeed it may be better to see it as a model of the fluid–solid transition phase, rather than of wound repair tissue, whatever its biological content. How can we seriously consider it a solid and how have we managed so long to focus on the collagen rather than the overwhelming water component? It is a tissue model which can contain less solid material that an cup of tea [2]. It is as if we propose to use our hot-air-ballooning experience as the basis for a Virgin Atlantic contract to model the operation of their new super-jumbo fleet. The balloon does provide a model of flight-but not as it relates to everyday human transport.

So, this review will analyse the idea that 3D collagen culture models are passing (have passed) through another reality-frontier, comparable to that of leaving plastic monolayer cultures. Importantly, the focus here will be on the cell mechanical and spatial environment, rather than molecular or compositional responses. After all, 3D collagen constructs represent above all the spatial-mechanical cell niche and only after that do they carry molecular signals.

Contraction: tiny cell forces applied to very soft gels

To continue the earlier analogy, hot-air-balloons are in fact a good way to learn about and to model *some of the basic* mechanisms of flight—and so it is with soft 3D collagen gels. Over 3–4 decades the study of Fibroblast Populated Collagen Lattices (soft FPLCs) have provided a good basic understanding of how cells react to, are modified by, and utilise small mechanical forces in their matrix, Many of our current concepts and approaches to cell-mechanics, tissue structure and cell-matrix spatial organisation trace their origins to the emergence of the FPLC contraction model, as described in detail by Bell et al. [3].

Interestingly, Bell and co-workers initially considered this to be a route to producing practical tissue equivalents, particularly skin. Indeed, they protected the technology and actively commercialised it for a tissue engineered skin equivalent, now in clinical use and known as 'Apligraf' [4,5]. At the same time the use of collagen matrix lattices as 3D culture research models became common. The resulting concepts and approaches have been analysed in a number of excellent reviews, from the perspective of the cell, molecular and mechanical processes which they might model [6–11]. There has been a clear shift, therefore, from the use of the collagen gel model to describe general fibroblast behaviour to one of understanding what happens to the *fibroblast-matrix continuum* under both external and local, cell-generated forces (Fig. 1).

Early studies established that the degree of geometric shrinking (contraction) of free-floating gels (assumed to be proportional to net cell force) was related to both the cell and collagen density. Contraction was most rapid in the first 12 hr, followed by exponential fall in rate to a near constant after perhaps