Rapid Fabrication of Living Tissue Models by Collagen Plastic Compression: Understanding Three-Dimensional Cell Matrix Repair *In Vitro*

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Abbreviations and Acronyms

ECM = extracellular matrix
FLS = fluid leaving surface
FPCLs = fibroblast-populated collagen lattices
HDFs = human dermal fibroblasts
PC = plastic compression

Objective: To produce biomimetic collagen scaffolds for tissue modeling and as tissue-engineered implants.

Approach: Control of collagen fibril material parameters in collagen hydrogel scaffolds by using plastic compression (PC), resulting in direct control of cell proliferation, cell migration, and cell-cell interaction.

Results: We were able to control the density of collagen in such scaffolds from between 0.2% and 30%, and controllably layer the fibrils in the Z-plane. Cell migration was observed in gels where a gradient of collagen density was present. In these gels, cells preferentially migrated toward the collagen-dense areas. Cell proliferation rates were measurably higher in dense collagen gels.

Innovation: The use of PC to control material properties of collagen hydrogels results in collagen scaffolds that are biomimetic. These collagen gels reproduce the relevant matrix-mechanical environment in which behavior is more representative of that found *in vivo*.

Conclusion: The material properties of native collagen type I gels can be engineered to match those found in tissues *in vivo* to elicit more biomimetic cell behavior.

INTRODUCTION

ENGINEERING OF REALISTIC, living three-dimensional (3D) tissues *in vitro* remains a challenging goal, yet there has never been a greater need to improve our game in this area. Tissue modeling, in general, is coming of age, with the gradual realization of how limited our monolayer (so-called two-dimensional [2D]) culture systems really are. This is nowhere more apparent than in stromal, or connective tissue function, pathology, and repair. Connective tissue cells, like those from dermis, fascia, and tendon are clearly more adapted to shuffling around underground (deep in the tissue fabric) rather than spreading over or skimming across the tissue surfaces,

with the sun on their backs. Each year more examples reach the literature in which cell behaviors totally switch when they can attach on all their surfaces to a relatively soft (compliant) extracellular matrix (ECM).

While it is obviously short-sighted to embark on engineering of 3D model tissue without simulating as much of the tissue composition and architecture as possible, it is easy to see why tissue complexity is intimidating. So, early generation tissue models have tended to be simplistic, leaving us content to persist with monolayers of cells, cells embedded in ultra-compliant hydrogels, or in random-structure sponges. However, after 20 years of engineering 3D tissues, we

should consider that the gauntlet is now down. The proposition is that we have been aiming too low, and still missing.

We can perhaps go back as far as the Elsdale and Bard model of fibroblasts contracting a 3D collagen gel (1972) to trace the sustained application of 3D native collagen systems for understanding wound repair mechanisms.¹ Since then, a host of variants of this system have been used with progressively more impressive claims as to what part of natural tissue function the behavior represents. Migration of fibroblasts or myofibroblasts through the bulk of such native collagen lattices is said to mimic host cell recruitment and distribution. Passage of cells, including fibroblasts, endothelial cells, smooth muscle cells, Schwann cells, and leucocytes across interfaces between adjacent collagen gels is thought to represent early blood vessel or nerve ingrowth and integration. Most of all, the cell generation of tractional forces within such gels, leading to their physical, geometric shrinkage, is said to mirror the processes of wound contraction and/or scar contracture. At the far extreme of this logic, it is suggested that cell-seeded 3D collagen lattices, floating freely and unrestrained in a culture medium (*i.e.*, under very low stress) mimic a contracting granulation tissue. By extension of this, restrained tethered gels, where collagen fibrils are under modest cell-generated tension are suggested to model contracture of a more mature scar tissue.²

Although widely accepted, this apparently reasonable interpretation suffers a potentially fatal problem, evident only where we calculate the total collagen content of such hyperhydrated collagen gels or lattices. Prepared from collagen solutions of typically 2–5 mg/mL, these gels start at collagen concentrations 0.2%–0.5% (or 99.8%–99.5% water). These are effectively unstable materials, in that they self-compress under their own weight when taken out of their liquid.³ Even after protracted and strong cell-mediated geometric contraction, this is unlikely to rise above 1%–2% collagen. Consequently, their relevance to tissues, such as granulation tissue and dermis, with collagen densities, an order of magnitude greater at 10%–20% collagen, must be doubtful, particularly, as so much of the model logic is dependent on the mechanical properties.

Clearly, materials comprising collagen fibril densities 10-fold less dense than their natural equivalent will not model their mechanical properties. Forces capable of contracting one will be far greater than the tiny cell-generated forces able to contract the other.⁴

Mechanical fidelity in our tissue models is important not only for understanding the effects of

external mechanical loads, but also cell migration or remodeling degradation of the bulk collagen material. To provide this mechanical minimum of comparable collagen densities would seem to be a basic requirement for credible models.

The mechanical properties of model tissues prepared by culturing cells (*e.g.*, dermal fibroblasts) in strong synthetic polymers (*e.g.*, polylactic or polyglycolic acids) tend to be stronger and more representative of native tissues. However, such synthetic polymers clearly are no model at all for a native fiber protein material of a natural connective tissue, and these mechanical properties may not be retained for longer periods of time. In addition, the resident cells take weeks/months (if ever) to deposit a collagen material with the density or mechanical properties approaching native tissues. Decellularized collagen tissues, such as fascia or dermis, are in wide use. These are clearly excellent mimics of the matrix element, but experimental cells can only realistically be grown over their collagen surfaces (a form of pseudo 3D system) as the collagen is so dense and (after processing) completely cell free. Inability to test the responses of interstitially seeded fibroblasts, growing deep in the collagen bulk (*i.e.*, in true 3D) would similarly, seem to be a fatal flaw.

Of these three apparently compromising flaws, the simplest to overcome now seems to be that of the poor collagen density of native collagen lattices. Recent technology involving collagen gel controlled compression, the plastic compression (PC) process, allows fibril density to be increased in a controlled manner. This can produce densities comparable to those of many native connective tissues, including dermis, but in a rapid, controllable manner.^{3–5} Originally developed for rapid fabrication of living tissues as clinical implants, PC is now seen as an advance in collagen engineering, which provides a route to making high fidelity experimental, diagnostic, and screening 3D collagen tissue models.⁶

CLINICAL PROBLEMS ADDRESSED

For accurate modeling of tissues, the density of ECM components in any given tissue is a critical component. This parameter is difficult to control and engineer *in vitro*, making the process of engineering tissue equivalents much harder. PC technology, particularly for collagen hydrogels, offers an avenue in which to control ECM density and configuration for successful engineering of matrix-rich tissue equivalents, as well as modeling tissues *in vitro*. A huge benefit of this technique is the limited effect on cell viability and the ability to

control spatial parameters of cell and matrix parameters in tissues.

APPROACH: FIXING THE DENSITY PROBLEM—AND ALL THAT FOLLOWS

When a solution of acid-soluble, monomer collagen is neutralized and brought to 37°C (in fact room temperature is sufficient), the monomer protein rapidly begins to aggregate in its native $\frac{1}{4}$ staggered manner to produce nanometer diameter fibrils. These fibrils elongate, and more slowly, grow in diameter, entwining to form a loose mesh, or lattice. From our perspective, we see a solution that becomes viscous and suddenly forms a weak gel. The process is similar in some ways to crystal formation, except in this case, the crystals are fibrils, tens of nanometers in diameter, and potentially many millimeters long. They form through all of the original liquid space/volume. This means that they effectively enclose or trap the original liquid volume. This is a key point.

First, this gelation process results in a hyperhydrated gel, in that the total protein mass (even assuming that it all gels) is 200–500 times less than the water/medium that it surrounds. The final gel is so insubstantial that it is unstable out of its supporting fluid and self compresses under its own weight. There is no greater significance to the final ratio of water to fibrils (hydration proportion) than the collagen concentration we selected to start with. Second, it means that all solids that are trapped when this lattice forms are very widely spaced. This solid phase includes the fibrils themselves, any cells seeded, inorganic particles, for example, hydroxyapatite mineral particles. These are almost always held at proportionally low density. They are restrained/retained by a nanoporous mesh (so proportional to diameter), which has minimal strength in compression.

A secondary feature of a process of natural protein aggregation is the enormous capacity for variability in the detail of this gelling stage. The temperature, pH, collagen purity (relating to the source of collagen), monomer–oligomer proportion, and protein completeness (atelo- or with telopeptides) all dramatically affect the speed, extent, pattern, and diameter of fibril formation, and so the hyperhydrated gel properties. Close technical control of this stage is essential if any useful models are to be made, as these are time-zero variations, which cell culture will only amplify.

For decades, this (now unattractive) position has been the finishing point of our models. However, while removal of fluid was known to be possible, it

has only recently become clear how a partial dehydration can be achieved rapidly without damaging the cells that are within that lattice. The process of PC is illustrated in Fig. 1 as a forced flow in one principle direction, in this case from top to bottom. In practice, fluid can be removed from either any or all of the gel faces, and in the later stages, the pattern of fluid loss in 3D can change in complex manners. The central controlling factor of outflow is governed by the primary fluid leaving surface (FLS), which retains particles and progressively restricts fluid outflow as it rapidly becomes denser (Fig. 2). Eventually, excessive packing of collagen (+/- cells) at the FLS, effectively blocks further fluid outflow, so self-limits the process. Typically, the end point material has the consistency of a moist cling-film; tough compliance, very thin (50–150 μm), but potentially a living sheet of seminatural connective tissue.

PC forces out much of the excess fluid tapped in the initial hyperhydrated gel, reducing the fluid content by 100–200-fold, typically from 99.8% to 85%–90%. This is comparable to native tissues. In this mode, the compressive deformation is almost entirely *plastic* (i.e., it does not return, recover, or re-swell after removal of the driving force). As described above, there are no physicochemical drivers to maintain this level of water content (e.g., protein polarity or osmotic effects): the water content was a function of gel casting. In practice, removal of this huge excess of fluid can be achieved by a range of methods, including blotting/capillary action, mechanical compression, centrifugation, and osmotic pressure.

Interestingly, the great surprise for workers who move from use of hyperhydrated to compressed gel models is that such an apparently simple process can alter so much of their utility and behavior. In effect, this comes from the new levels of control (of properties, content, 3D μ -architecture, and mechanics), reproducibility (e.g., simultaneous multiwell compression), and rapid flexibility it gives. This rapid flexibility for customizing of model tissues comes particularly with the use of multilayers (heterogeneous or homogeneous layers), by stacking, rolling, or serial compression of PC sheets (Figs. 1 and 3). The first and most important point of control is that collagen density (as well as cells and other particles) can be predetermined by control of the amount of fluid removed. The second control point is where that fluid is primarily taken from—that is, the FLS. The FLS dominates control of the 3D μ -structure, alignment, and topography.⁷ Where additional matrix components have been incorporated into

Figure 1. Schematic of plastic compression (PC) to form dense sheets, which can be layered to produce increasingly complex tissues. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 2. Formation of fluid leaving surface (FLS) during PC process, and the subsequent layering the FLS undergoes. (a) Cells trapped within a hyper-hydrated collagen gel. (b) It is possible to see the first dense FLS layer being made. (c) As the PC process continues, additional layers of dense collagen are formed. (d) Additionally, cells become trapped within these layers. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

Figure 3. Spiralled PC collagen sheets with alternating extracellular matrix proteins. These are ideally tissue specific. **(a)** PC collagen sheet rolled to give spiral configuration. **(b)** Collagen sheet overlaid with a fibronectin sheet, then spiraled for use as a neural tissue-engineered construct. **(c)** Collagen sheet sprinkled with hydroxyapatite particles, and then spiraled for formation of bone tissue-engineered constructs. Reprinted with permission from Brown *et al.*³ To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

engineered constructs, and even cocompressed with collagen gels, we have not yet ascertained the effect of this compression on other materials (Fig. 3). Given the complex matrix composition of most tissues within the body, controlling the density of each matrix component will need to be addressed.

The PC process itself is deceptively simple, but its detail dictates the manner and extent to which 3D PC models can be mathematically predicted and able to model natural tissues. The variability of models using hyperhydrated collagen gels is only now becoming clear, in which case very little prediction or calculation of structure or composition was possible. The point of PC collagen models is that they start with a constant, predefinable set of parameters, with their cells already in place. It is then completely simple to start experimental systems from a clean, precise $\text{TIME}_{\text{zero}}$ by simply waking up the seeded cells with standard culture conditions. The total PC process takes only 30–45 min and requires absolutely no cell participation, so it negates this notorious source of variability.

RESULTS

Examples of cell–matrix model systems

By culturing cells in a more biomimetic collagen matrix, by using PC to create tissue-like dense collagen materials, we have been able to influence

cell behavior in three dimensions. In particular, we have measured cell parameters, including proliferation, migration, and signaling and seen marked differences in cell behavior in collagen matrices of different density, with the biomimetic density conferring more biomimetic responses by cells.

Below we highlight some of these findings.

Cell proliferation. Cell proliferation is higher in dense PC collagen gels compared to standard low-density hydrogels. In studies where the collagen density of gels was varied, a clear difference was observed in cell proliferation rates.⁸ In gels of 0.7% collagen, there was an 87% increase in cell number within 48 h of culture, this was reduced to a 25% increase in gels of 0.4% density, and at 0.2% density no proliferation was measured.⁸ This behavior explains the previous (hyperhydrated gel) finding that cells largely do not proliferate in free-floating gels, but will start to divide after a lag period in tethered gels/lattices. Importantly, this study showed that quiescent cells in free-floating gels were stimulated to divide almost at once if the gel was compressed, at any point in its culture. As in natural tissues, resident fibroblasts divide rapidly until they reach an apparently predetermined cell density, comparable to confluence in monolayer culture.

Cell migration. Cell migration was predictably affected by collagen density and mechanical stiffness. PC constructs were made by casting a non-rectangular starting gel and compressing this in a manner that produces a gradient of collagen density across its length⁹ (as shown in Fig. 4). This gradient gel is formed by effectively setting a

Figure 4. Production of gradient PC scaffolds using a wedge-shaped mold. Following setting, the hydrogel is removed and compressed, resulting in an even thickness gel with a graduated density of collagen. To see this illustration in color, the reader is referred to the web version of this article at www.liebertpub.com/wound

standard collagen hydrogel within a wedge-shaped mold. This produces a gradient of total collagen along the length of this gel.⁹ Once set, the entire collagen gel is plastically compressed horizontally to express its excess fluid as normally, but leaving a much greater density of collagen at the thick, as opposed to the thin end of the gel. The resulting construct has a uniform thickness height, but a density gradient of collagen (Fig. 4). Notably, the rate of change and extent of the gradient can be determined by the casting angle of the initial gel and, in fact, nonlinear gradients are possible by casting into molds with a curved base. The collagen density in these gradients was not measured directly. Rather, the material stiffness was measured, as the direct consequence of collagen content, and this increased in proportion to the predicted increase in collagen density. At the low density/soft end, Young's modulus was $1,057 \pm 487$ kPa, increasing to $2,305 \pm 693$ kPa at the high density/stiff end.⁹

Along with the better-known cell migration drivers and guidance cues, such as chemotaxis, chemokinesis, and topography, the response of cells to increasing substrate stiffness has been described and termed duro-taxis. Duro-taxis is not a simple effect to demonstrate, especially using natural materials. Consequently, its mechanisms, although discussed previously, is not well understood.⁹ However, dermal fibroblasts were found to migrate to and accumulate preferentially at the stiffer end of the scaffold over a 6-day culture period. Other groups have suggested that cell behavior is more biomimetic when cells are cultured in stiffer matrices, in the case of stem cell differentiation, relating cell fate to the stiffness of the parent tissues, varying from brain (to neurites) at the soft end to bone (to osteoblasts) at the hard extreme.¹⁰ Cell-generated tensional homeostasis is also a critical factor in cell responses, so the mechanical environment in which a cell resides, and then the cell response to this mechanical environment in terms of endogenous tension generation, greatly influences cell behavior.¹¹ Perhaps, the simplest hypothesis to explain duro-taxis, is one based on the mechanics, as opposed to one based on the biology. The cell cytoskeleton exerts isometric tension on the material into which it is embedded, and is able to deform this matrix. Where the matrix is stiffer, cell movement is more likely, compared to materials that are compliant, where deformation and/or remodeling is more likely.¹² Newtonian mechanics, then, says that cells will move faster on and toward harder materials.

Chemical gradient. Chemical gradient diffusion in PC collagen gels has implications for cell

migration and aggregation in response to this. One of the advantages of using PC scaffolds as a starting point for tissue fabrication is the ability to control the cellular microenvironment by customizing parameters, such as the diffusive properties of the scaffold and/or the seeding cell density. Indeed, previous work using PC scaffolds has shown that O₂ tension in the core of *in vitro*-cultured 3D collagen constructs is controlled by adjusting the seeding cell density and therefore cell O₂ consumption.¹³ For example, seeding constructs with human dermal fibroblasts (HDFs) at high density (23.2×10^6 cells/mL) resulted in rapid reduction of core O₂ tension toward plateau values of ~ 25 mm Hg (3.2%), that is, in the low end of the physiological hypoxic range. Importantly, exposure of core HDFs to hypoxia was accompanied by increasing upregulation of VEGF gene expression up to 8 days, while this response was spatially specific (higher expression in the core vs. mid and surface).¹³ Work from this group and others has shown that multiple cell types (e.g., dermal fibroblasts, bone marrow-derived stromal cells, and vascular smooth muscle cells) respond to physiological hypoxia by upregulating angiogenic signaling in PC scaffolds. The spatial and temporal control of this angiogenic signaling is easily controlled in PC scaffolds by seeding cells at specific cell densities.

It is known that long-term exposure of cells to hypoxia can be detrimental to cell viability; however, culturing cells at low levels of physiological hypoxia can be employed as a stimulus for triggering the upregulation of the complete angiogenic cascade.¹³ The ability to spatially localize hypoxia-induced signaling cells within 3D PC scaffolds provides a powerful tool for engineering *in vitro* models of angiogenesis. Notably, the upregulation of angiogenic factors by depots of HDFs has been shown to result in the migration of endothelial cell toward the source signaling, as well as aggregation of these cells to form capillary-like structures.¹⁴

Modeling matrix remodeling. Three-dimensional models of cell-matrix remodeling have long been associated with the conventional collagen gel culture system of fibroblast-populated collagen lattices (FPCLs). This cell-mediated remodeling has two aspects: (1) compositional and (2) spatial remodeling of the material architecture. In conventional FPCLs, most interest has focused on geometric shrinkage, which is termed contraction.^{15,16} As discussed previously, it is unsurprising, given the increased stiffness of PC collagen constructs (up to 2 MPa)⁴ and the minimal forces generated by fibroblasts that similar levels of

contraction are not seen for fibroblast-PC collagen.³ However, modest changes of the construct shape are seen over days and weeks in culture, but these are associated with internal changes in the structure. Histologically, the initially dense collagen layers gradually take on a lacey appearance, apparently as the resident fibroblasts migrate over the internal layers, removing, and replacing the original dense collagen. It is microscopically clear the cells slowly degrade collagen from the lamellar surfaces. Additionally, it is implied from the simultaneous increase in mechanical strength, that this is replaced by deposition of a more efficient matrix.³ It is also possible that these same cells produce a gradual contracture,* a permanent matrix shape change by the ratchet mechanism proposed and identified previously.^{16,17} Observations so far, then, would suggest that the small, slow shape changes seen for PC collagen constructs are equivalent to the contractures seen in clinical scars.

The second important remodeling feature of PC collagen (in contrast to hyperhydrated FPCLs) is their substantial, indeed surprising stability to fibroblast, and other cell degradation. Despite the PC collagen being a perfectly natural substrate for fibroblast matrix metalloproteinase enzyme systems, including collagenases, it is largely intact after as long as 5 weeks in culture.³ This may of course be due to a balanced deposition of new collagen onto the old. This effect is even more pronounced after *in vivo* implantation,¹⁸ for up to 5 weeks, when the PC construct and cells remained intact, although again remodeled with fresh collagen deposition.

Cell invasion. The key observation in both *in vivo* and *in vitro* systems using PC collagen has been that all invading cells so far studied appear to avoid whole-sale invasion and degradation of the bulk dense matrix. Rather, cell migration is enhanced in these materials, and found to be prominent along any preformed surfaces, such as clefts or channel defects between layers. This was most obvious where spiraled-layer PC constructs were implanted and host cells invaded following the (easier) spiral path from edge to core, rather than a direct radial invasion, that is, through layers which would need to be remodeled.¹⁸ This is a typical mirror of *in vivo* behavior of tissue remodeling fibroblasts meaning that the tissue is remodeled in

its original image (*i.e.*, the composition is replaced, but the architecture remains). This was most striking in the *in vivo* model using spiraled PC constructs, which retained their original spiral structure (completely foreign to the local tissue) even after 5 weeks. This clearly models the manner in which skin grafts from the back to the face still resemble back-skin many decades after surgery. In other words, the PC spiral collagen can mimic natural remodeling, where the matrix fabric is replaced, but without loss of μ -architecture: that is, there is a 3D structural memory retained by the local tissue matrix.

O₂ tensions. O₂ tensions around cells *in vivo* are much lower than those in which cells are cultured in standard tissue culture. *In vivo* cells reside between 2% and 10% O₂, whereas in 2D culture, the O₂ tension is almost 21%. By culturing cells within a 3D scaffold, the O₂ tension is lowered, sometimes to the detriment of cells when the levels fall below 1%. In cultures where 3D PC sheets were spiraled to give gross, mm-scale structure, at high densities of dermal fibroblasts, levels of O₂ tension did not fall below 2% over 10 days in culture (Fig. 5a, d).¹⁴ This, in part, is due to the fact that the diffusion coefficient of O₂ through these PC

Figure 5. (a) Formation of nutrient and O₂ consumption gradients in spiraled three-dimensional (3D) collagen constructs. (b) Scanning electron micrograph showing addition of phosphate-based dissolving glass fibers. (c) Schematic showing channeled construct produced following dissolution of fibers. (d) O₂ graph showing decrease in core O₂ tension due to cell consumption within a 3D construct. Where channels were added to the construct, following dissolution there is a gradual increase in O₂ tension within the core. Adapted from Cheema *et al.*²⁰

*It is important here to distinguish between models of contraction, where cell forces temporarily deform the matrix and the contracture of scar and Dupuytren's contracture. In contracture, the matrix material is permanently, physically shortened (by collagen remodeling/crosslinking), but in contraction, elimination of the cell force (by cytoskeletal disruption) allows the matrix shortening to recover.

constructs is relatively high, and the O₂ consumption gradients which are formed are sustained by this relatively high-diffusion coefficient.^{19,20}

In 3D tissue models, there is a heterogeneous distribution of O₂, which is higher at the surface than the core.¹³ This O₂ distribution is cell specific and ultimately determined by the rate of O₂ consumption, which differs between cell types.

The introduction of soluble phosphate glass fibers into 3D PC constructs involved laying parallel fibers over a PC collagen sheet and rolling into a spiral (Fig. 5b).²¹ These phosphate glass fibers dissolve over 24 h, leaving patent, full length channels. Where channels were introduced into these 3D spiraled constructs, the core O₂ tension increased near to ambient O₂ tension within the incubator (Fig. 5). Channeling increased core O₂ tension by taking culture medium deep into the constructs, and also provided the framework for vascular or neural ingrowth, *in vitro* or *in vivo*. This is an important strategy for improving how we model the process of angiogenesis *in vitro* and identify methods to accelerate vascularization of implants *in vivo*.²¹

This reflects the idea that the native 3D environment around cells *in vivo* is not recapitulated by standard 2D culture, and so many elements of cell behavior are not accurately mimicked. Using experimental data and simulation, we have found that the O₂ consumption rate of cells in PC collagen model tissues more closely mimics that, and other behaviors, of cells *in vivo*,²² emphasizing how 3D biomimetic constructs can affect multiple cell behaviors.

DISCUSSION

The process of PC in principle is very simple, in that it expels excess fluid from hyperhydrated collagen gels. In this respect, the main parameters altered by this process are collagen density and alignment into layers. This PC collagen has the benefit of more closely matching tissue collagen levels, particularly matrix-rich tissues. In a series of studies, we have demonstrated that the PC fabrication technique gives additional control of matrix properties, such as the ability for this collagen to be layered, spiraled, and interspersed with other matrix components and to spatially configure different cell populations in three dimensions. In addition to this, key cell responses in PC collagen more closely mimic equivalent cell behaviors *in vivo*. This is likely to be a result of the more complex 3D biomimetic microenvironment provided by the PC collagen.

Complex tissues need to be engineered from simple component parts where the sequence, orientation, and content of 3D layers provide much of the model

KEY FINDINGS

- Control of collagen density in tissue-engineered scaffolds is critical for biomimetic tissue engineering, and PC fulfils this role.
- We have observed the biomimetic cell responses in PC scaffolds, comparable to other 3D scaffolds.
- Addition of ECM components, synthetic materials, and cell-specific layers have resulted in architectural complexity within PC scaffolds to gradually build tissue complexity.

complexity we require. This reflects an emerging view that rapid, controllable assembly of 3D tissue-like layers will lead to completely new forms of model tissues for research, diagnosis, screening, and testing. This can presently be achieved by variants of matrix or cell layer engineering, 3D ink-jet bioprinting, or some forms of electrospinning.^{3,23,24}

INNOVATION

Current strategies used for engineering tissue models and tissue equivalents do not tackle the issue that tissue collagen density and matrix density are not adequately reproduced and not biomimetic. PC of collagen gels is a controlled method to increase collagen density, with the benefit of layering the collagen in the Z-plane. PC collagen is more biomimetic, and as such tissue models will be more representative of native tissue. PC collagen will also likely yield better results as a tissue-engineered equivalent compared to many other collagen materials.

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AUTHOR DISCLOSURE AND GHOSTWRITING

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ABOUT THE AUTHORS

Dr. Umber Cheema holds a BBSRC David Phillips fellowship, based on understanding how to engineer biomimetic 3D tissue models. Her work includes the development of models for skeletal muscle and other musculoskeletal tissues using collagen

type I gels. Her research also focuses on controlling elements of collagen architecture to build biomimetic tissues *in vitro*. In particular, mechanisms to control collagen fibril alignment, diameter, and density, and the introduction of architectural features (channels) to provide the basis of vascular networks in tissue-engineered constructs. **Professor Robert Brown** directs the UCL Centre for Tissue Regeneration Sciences. Early research in collagen and angiogenesis led him to establish the UCL Tissue Repair Centre with Professor McGrouther, feeding basic science to the work of Plastic and Reconstructive Surgery.

This produced new, internationally leading models for cell–matrix loading and scarring. Over 10 years, their purpose evolved from understanding the 3D models to making them into engineered graft tissues. As UCL's first professor of tissue engineering, he discovered a new approach to rapid layer-fabrication of living tissues using collagen PC. This has developed into many forms of machine-fabrication of tissues (cornea, skin, tendon, nerve, urothelium) and commercial drug testing kits through >210 peer-reviewed publications and patents. He teaches and promotes interdisciplinary research internationally.

REFERENCES

1. Elsdale T and Bard J: Collagen substrata for studies on cell behavior. *J Cell Biol* 1972; **54**: 626.
2. Eastwood M, Mudera VC, McGrouther DA, and Brown RA: Effect of precise mechanical loading on fibroblast populated collagen lattices: morphological changes. *Cell Motil Cytoskeleton* 1998; **40**: 13.
3. Brown RA, Wiseman M, Chuo CB, Cheema U, and Nazhat SN: Ultrarapid engineering of biomimetic materials and tissues: fabrication of nano- and microstructures by plastic compression. *Adv Funct Mat* 2005; **15**: 1762.
4. Abou-Neel EA, Cheema U, Knowles JC, Brown RA, and Nazhat SN: Use of multiple unconfined compression for fine control of collagen gel scaffold density and mechanical properties. *Soft Matter* 2006; **2**: 986.
5. Bitar M, Salih V, Brown RA, and Nazhat SN: Effect of multiple unconfined compression on cellular dense collagen scaffolds for bone tissue engineering. *J Mater Sci Mater Med* 2007; **18**: 237.
6. Braziulis E, Diezi M, Biedermann T, Pontiggia L, Schmucki M, Hartmann-Fritsch F, Luginbühl J, Schiestl C, Meuli M, and Reichmann E: Modified plastic compression of collagen hydrogels provides an ideal matrix for clinically applicable skin substitutes. *Tissue Eng Part C Methods* 2012; **18**: 464.
7. Alekseeva T, Hadjipanayi E, Abou Neel EA, and Brown RA: Engineering stable topography in dense bio-mimetic 3D collagen scaffolds. *Eur Cell Mater* 2012; **23**: 28.
8. Hadjipanayi E, Mudera V, and Brown RA: Close dependence of fibroblast proliferation on collagen scaffold matrix stiffness. *J Tissue Eng Regen Med* 2009; **3**: 77.
9. Hadjipanayi E, Mudera V, and Brown RA: Guiding cell migration in 3D: a collagen matrix with graded directional stiffness. *Cell Motil Cytoskeleton* 2009; **66**: 121.
10. Tse JR and Engler AJ: Stiffness gradients mimicking *in vivo* tissue variation regulate mesenchymal stem cell fate. *PLoS One* 2011; **6**: e15978.
11. Chicurel ME, Chen CS, and Ingber DE: Cellular control lies in the balance of forces. *Curr Opin Cell Biol* 1998; **10**: 232.
12. Brown RA: Cytomechanics in connective tissue repair and engineering. In: *Tissue Repair Contraction and the Myofibroblast*, edited by Chaponnier C, Desmouliere A, and Gabbiani G. Georgetown: Landes Bioscience, 2006, pp. 7–24.
13. Cheema U, Brown RA, Alp B, and Macrobert AJ: Spatially defined oxygen gradients and vascular endothelial growth factor expression in an engineered 3D cell model. *Cell Mol Life Sci* 2008; **65**: 177.
14. Hadjipanayi E, Brown RA, Mudera V, Deng D, Liu W, and Cheema U: Controlling physiological angiogenesis by hypoxia-induced signalling. *J Control Release* 2010; **146**: 309.
15. Grinnell F: Fibroblast biology in three-dimensional collagen matrices. *Trends Cell Biol* 2003; **13**: 264.
16. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, and Brown RA: Myofibroblasts and mechano-regulation of connective tissue remodelling. *Nat Rev Mol Cell Biol* 2002; **3**: 349.
17. Marenzana M, Wilson-Jones N, Mudera V, and Brown RA: Origins and regulation of tissue tension: first identification of *in vitro* tension fixation in a collagen material. *Exp Cell Res* 2006; **312**: 423.
18. Mudera V, Morgan M, Cheema U, Nazhat SN, and Brown RA: Ultra-rapid engineered collagen constructs tested in an *in vivo* nursery site. *J Tissue Eng Regen Med* 2007; **1**: 192.
19. Cheema U, Rong Z, Kirresh O, Macrobert AJ, Vadgama P, and Brown RA: Oxygen diffusion through collagen scaffolds at defined densities: implications for cell survival in tissue models. *J Tissue Eng Regen Med* 2012; **6**: 77.
20. Cheema U, Alekseeva T, Abou-Neel EA, and Brown RA: Switching off angiogenic signalling: creating channelled constructs for adequate oxygen delivery in tissue engineered constructs. *Eur Cell Mater* 2010; **20**: 274.
21. Kaully T, Kaufman-Francis K, Lesman A, and Levenberg S: Vascularization—the conduit to viable engineered tissues. *Tissue Eng Part B Rev* 2009; **15**: 159.
22. Streeter I and Cheema U: Oxygen consumption rate of cells in 3D culture: the use of experiment and simulation to measure kinetic parameters and optimise culture conditions. *Analyst* 2011; **136**: 4013.
23. Haraguchi Y, Shimizu T, Sasagawa T, Sekine H, Sakaguchi K, Kikuchi T, Sekine W, Sekiya S, Yamato M, Umezumi M, and Okano T: Fabrication of functional three-dimensional tissues by stacking cell sheets *in vitro*. *Nat Protoc* 2012; **7**: 850.
24. Luo CJ, Stoyanov SD, Stride E, Pelan E, and Edirisinghe M: Electrospinning versus fibre production methods: from specifics to technological convergence. *Chem Soc Rev* 2012; **41**: 4708.