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Building stable anisotropic tissues using cellular collagen gels

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Combining cellular self-alignment within tethered collagen gels with stabilization through subsequent removal of interstitial fluid has yielded a new process for the fabrication of aligned cellular biomaterials. This commentary discusses the generation of engineered neural tissue for peripheral nerve repair using this combination of techniques, providing additional insight into the rationale underpinning the approach. By describing the potential benefits of using cell and matrix interactions to organize 3D hydrogels that can be stabilized to form tissue-like constructs, the article aims to highlight the potential for the approach to be used in the generation of a wider range of functional replacement tissues.

Keywords: collagen, hydrogel, regenerative medicine, tissue engineering, nerve, anisotropy, Schwann cell, biomaterials, alignment

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The recent publication "Engineered neural tissue for peripheral nerve repair" in *Biomaterials* by Georgiou et al.[1] described a new method for generating aligned cellular biomaterials, which combined cellular self-alignment in collagen gels with a stabilization step involving removal of interstitial fluid. The focus in that work was to build engineered neural tissue (EngNT) for peripheral nerve repair, but it is clear that the approach could be applied more widely to the production of various artificial tissues and organs where anisotropic structures are desired. The aim of this Commentary is to provide some additional insight into the rationale underpinning this combination of techniques and to highlight the potential for usage beyond the nerve repair field.

Alignment of cells and matrix is a feature commonly observed within tissues, particularly those of the musculoskeletal system and some parts of the nervous system. This organized architecture can be critical to function, with scarring and altered mechanical function a common consequence of repairs that fail to recreate organized tissue structures.[2] Engineering aligned cellular structures in vitro is therefore a common aim within the tissue engineering and regenerative medicine research community, and a range of approaches have been explored including the use of gradients of chemical and mechanical properties, electrical and magnetic fields, mechanical loading of cellular constructs, and numerous anisotropic biomaterial scaffolds (for reviews see refs. 3 and 4). This latter approach is commonly employed by tissue engineers to confer alignment upon cells through restricting or guiding cellular adherence and spreading on the surfaces of structured constructs.[5-9]

Building anisotropic cellular constructs using a traditional tissue engineering approach requires manufacture of organized 3D scaffolds containing channels, fibers, pores, or other topographical features, often combined with chemical modification of surfaces to facilitate cell adhesion. The widespread use of synthetic polymers for this purpose enables a high degree of control in terms of production consistency and offers powerful opportunities for engineering elaborate structures and patterns at the micro- and nano-scale. However, this approach also requires a cell-seeding step which may be challenging in an elaborate 3D construct, cells may be subjected to undesirable spatial and mechanical cues through attachment to stiff material surfaces,[2,10] and because there is little scope for cell-mediated remodelling of synthetic matrices in vivo scaffolds must be completely resorbed.

The approach reported by Georgiou et al. resulted in aligned cells distributed evenly throughout a stable aligned matrix made from native type I collagen. The cells and matrix effectively organized each other into their orientated aligned structure via integrin-mediated interactions and cytoskeletal contraction. The contraction of cells (in this case Schwann cells) applied strain to their local 3D matrix environment, causing cells and collagen fibrils to become aligned in response to the resulting tension that developed longitudinally in the tethered rectangular gels.[11] It is important to note that the alignment of collagen fibrils and the orientation of the cells occurred simultaneously through the intimate coupling of cell and matrix movements, driven entirely by cell-generated forces acting within a constrained compliant collagen gel.

This phenomenon of cellular self-alignment in tethered collagen gels is well established.[12-14] However, while it has provided a useful means by which to study the effects of cellular alignment in vitro,[15] previous attempts to exploit it as a means to generate anisotropic cellular constructs for tissue repair have been limited by the need for continued tethering and the inherently low strength of the collagen hydrogels.[2] We demonstrated the efficacy of using tethered self-aligned Schwann cells in a collagen gel for nerve repair by generating and implanting the constructs within modified silicone tubes,[16] but the challenges involved in scaling up and translating an approach in which the delicate tethering of collagen gels must be maintained continue to be considerable. More generally, collagen hydrogels have been used as cell-delivery substrates in tissue engineering for many years,[17-19] but poor mechanical strength tends to preclude their use as principal components in the fabrication of more organized tissue structures.[2,20]

An elegant solution to the problem of how to convert weak "hyper-hydrated" collagen gels into robust tissue-like collagen constructs without damaging resident cells was published by Brown et al. in 2005.[21] By removing much of the interstitial fluid, cell and collagen density could be increased, resulting in strong stable constructs that have subsequently been used in a wide range of tissue engineering applications.[22-27] Georgiou et al. applied this stabilization technique to Schwann cell-populated collagen gels after cellular self-alignment, increasing cell and collagen density to a sufficient extent that tethering could be removed from the gels without subsequent loss of cell and matrix alignment. The resulting sheet of stable aligned cellular material guided regenerating neurons and was robust enough to withstand being rolled and wrapped to form an implantable construct. The use of native type I collagen and the avoidance of synthetic support scaffolds and chemical cross linking facilitated integration with host peripheral nerve tissue.

This combination of collagen gel manipulation technologies presents a potentially valuable opportunity for the construction of anisotropic tissue constructs that could be used in a wide range of different scenarios. In addition to our use in peripheral nerve where a construct containing aligned Schwann cells within an aligned collagen matrix is an obvious candidate for promoting neural repair, there are likely to be applications elsewhere in the nervous system, particularly in repairing the aligned tracts that can be damaged in spinal cord injury. We have shown previously that aligned astrocytes within a collagen gel can support and guide neuronal regeneration in vitro, and that aligned astrocyte gels can be stabilized through removal of interstitial fluid.[15] It will be interesting to explore in vivo whether this approach can yield implantable cellular materials for CNS repair, incorporating astrocytes or therapeutic cells suitable for use in a CNS environment.[28]

Beyond the nervous system, it would be useful to investigate whether the same approach could be applied to regeneration of some of the numerous other tissues where anisotropy and cellular organization are critical to function. Obvious candidates are musculoskeletal and connective tissues[20] and myocardium[29] as well as other structures where current techniques tend to involve shaping biomaterial scaffolds for cell delivery rather than using cell-mediated matrix organization in the fabrication of replacement tissues.

This initial example of EngNT will be developed further, in particular through the incorporation of therapeutically relevant human cells, clinically acceptable collagen sources and improved production processes to facilitate regulatory approval, scale-up, commercialisation, and translation to the clinic. The stabilization process can now be performed using commercially available absorbers (RAFT™, TAP Biosystems) and a range of stem cell-derived Schwann cell replacements are under investigation.

As techniques for manipulating collagen gels through directing cellular self-organization and through control of interstitial fluid proportion become better understood, it is likely that they will provide new ways to assemble a wider range of structures. In the study by Georgiou et al. simple rectangular sheets of EngNT were rolled to form rods, but there is much scope for assembling more elaborate tissue and organ structures through the use of multi-layering, formation of tubes, folding and shaping of sheets, embossing features onto surfaces and incorporation of depots of factors or supplementary matrix materials.

In summary, the Georgiou et al. study demonstrates a new approach for the fabrication of collagen hydrogels with tissue-like physical properties and anisotropy at both the cell and fibril level. It marks another advance in the rapidly progressing area of research that seeks to exploit understanding of cell and matrix behavior in hydrogels in order to engineer functional replacement tissues. Collagen hydrogels have been used widely for many decades as cellular substrates, and as new techniques for improving their performance emerge it is likely they will continue to serve as key tissue engineering tools for the construction of artificial tissues and organs in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Georgiou M, Bunting SC, Davies HA, Loughlin AJ, Golding JP, Phillips JB. Engineered neural tissue for peripheral nerve repair. Biomaterials 2013; 34:7335-43; PMID:23834895; http://dx.doi.org/10.1016/j.biomaterials.2013.06.02
2. Brown RA, Phillips JB. Cell responses to biomimetic protein scaffolds used in tissue repair and engineering. Int Rev Cytol 2007; 262:75-150; PMID:17631187; http://dx.doi.org/10.1016/S0074-7696(07)62002-

3. Park H, Cannizzaro C, Vunjak-Novakovic G, Langer R, Vacanti CA, Farokhzad OC. Nanofabrication and microfabrication of functional materials for tissue engineering. Tissue Eng 2007; 13:1867-77; PMID:17518744; http://dx.doi.org/10.1089/ten.2006.019
4. Lim JY, Donahue HJ. Cell sensing and response to micro- and nanostructured surfaces produced by chemical and topographic patterning. Tissue Eng 2007; 13:1879-91; PMID:17583997; http://dx.doi.org/10.1089/ten.2006.015
5. Bettinger CJ, Langer R, Borenstein JT. Engineering substrate topography at the micro- and nanoscale to control cell function. Angew Chem Int Ed Engl 2009; 48:5406-15; PMID:19492373; http://dx.doi.org/10.1002/anie.20080517
6. Bozkurt A, Deumens R, Beckmann C, Olde Damink L, Schügner F, Heschel I, Sellhaus B, Weis J, Jahnen-Dechent W, Brook GA, et al. In vitro cell alignment obtained with a Schwann cell enriched microstructured nerve guide with longitudinal guidance channels. Biomaterials 2009; 30:169-79; PMID:18922575; http://dx.doi.org/10.1016/j.biomaterials.2008.09.01
7. Kalbermatten DF, Erba P, Mahay D, Wiberg M, Pierer G, Terenghi G. Schwann cell strip for peripheral nerve repair. J Hand Surg Eur Vol 2008; 33:587-94; PMID:18977829; http://dx.doi.org/10.1177/175319340809075
8. Lietz M, Dreesmann L, Hoss M, Oberhoffner S, Schlosshauer B. Neuro tissue engineering of glial nerve guides and the impact of different cell types. Biomaterials 2006; 27:1425-36; PMID:16169587; http://dx.doi.org/10.1016/j.biomaterials.2005.08.00
9. Gerberich BG, Bhatia SK. Tissue scaffold surface patterning for clinical applications. Biotechnol J 2013; 8:73-84; PMID:23193104; http://dx.doi.org/10.1002/biot.20120013
10. East E, Golding JP, Phillips JB. A versatile 3D culture model facilitates monitoring of astrocytes undergoing reactive gliosis. J Tissue Eng Regen Med 2009; 3:634-46; PMID:19813215; http://dx.doi.org/10.1002/term.20
11. Phillips JB, Brown R. Micro-structured materials and mechanical cues in 3D collagen gels. Methods Mol Biol 2011; 695:183-96; PMID:21042973; http://dx.doi.org/10.1007/978-1-60761-984-0_1
12. Eastwood M, Mudera VC, McGrouther DA, Brown RA. Effect of precise mechanical loading on fibroblast populated collagen lattices: morphological changes. Cell Motil Cytoskeleton 1998; 40:13-21; PMID:9605968; http://dx.doi.org/10.1002/(SICI)1097-0169(1998)40:1<13::AID-CM2>3.0.CO;2-
13. Mudera VC, Pleass R, Eastwood M, Tarnuzzer R, Schultz G, Khaw P, McGrouther DA, Brown RA. Molecular responses of human dermal fibroblasts to dual cues: contact guidance and mechanical load. Cell Motil Cytoskeleton 2000; 45:1-9; PMID:10618162; http://dx.doi.org/10.1002/(SICI)1097-0169(200001)45:1<1::AID-CM1>3.0.CO;2-
14. Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 2002; 3:349-63; PMID:11988769; http://dx.doi.org/10.1038/nrm80
15. East E, de Oliveira DB, Golding JP, Phillips JB. Alignment of astrocytes increases neuronal growth in three-dimensional collagen gels and is maintained following plastic compression to form a spinal cord repair conduit. Tissue Eng Part A 2010; 16:3173-84; PMID:20649441; http://dx.doi.org/10.1089/ten.tea.2010.001
16. Phillips JB, Bunting SC, Hall SM, Brown RA. Neural tissue engineering: a self-organizing collagen guidance conduit. Tissue Eng 2005; 11:1611-7; PMID:16259614; http://dx.doi.org/10.1089/ten.2005.11.161
17. Bell E, Ivarsson B, Merrill C. Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro. Proc Natl Acad Sci U S A 1979; 76:1274-8; PMID:286310; http://dx.doi.org/10.1073/pnas.76.3.127
18. Wallace DG, Rosenblatt J. Collagen gel systems for sustained delivery and tissue engineering. Adv Drug Deliv Rev 2003; 55:1631-49; PMID:14623405; http://dx.doi.org/10.1016/j.addr.2003.08.00
19. Sabolinski ML, Alvarez O, Auletta M, Mulder G, Parenteau NL. Cultured skin as a 'smart material' for healing wounds: experience in venous ulcers. Biomaterials 1996; 17:311-20; PMID:8745328; http://dx.doi.org/10.1016/0142-9612(96)85569-
20. Brown RA. In the beginning there were soft collagen-cell gels: towards better 3D connective tissue models? Exp Cell Res 2013; 319:2460-9; PMID:23856376; http://dx.doi.org/10.1016/j.yexcr.2013.07.00
21. Brown RA, Wiseman M, Chuo CB, Cheema U, Nazhat SN. Ultrarapid engineering of biomimetic materials and tissues: Fabrication of nano- and microstructures by plastic compression. Adv Funct Mater 2005; 15:1762-70; http://dx.doi.org/10.1002/adfm.20050004
22. Levis HJ, Massie I, Dziasko MA, Kaasi A, Daniels JT. Rapid tissue engineering of biomimetic human corneal limbal crypts with 3D niche architecture. Biomaterials 2013; 34:8860-8; PMID:23968855; http://dx.doi.org/10.1016/j.biomaterials.2013.08.00
23. Levis HJ, Peh GS, Toh KP, Poh R, Shortt AJ, Drake RA, Mehta JS, Daniels JT. Plastic compressed collagen as a novel carrier for expanded human corneal endothelial cells for transplantation. PLoS One 2012; 7:e50993; PMID:23226443; http://dx.doi.org/10.1371/journal.pone.005099
24. Levis HJ, Brown RA, Daniels JT. Plastic compressed collagen as a biomimetic substrate for human limbal epithelial cell culture. Biomaterials 2010; 31:7726-37; PMID:20674002; http://dx.doi.org/10.1016/j.biomaterials.2010.07.01
25. Braziulis E, Diezi M, Biedermann T, Pontiggia L, Schmucki M, Hartmann-Fritsch F, Luginbühl J, Schiestl C, Meuli M, Reichmann E. Modified plastic compression of collagen hydrogels provides an ideal matrix for clinically applicable skin substitutes. Tissue Eng Part C Methods 2012; 18:464-74; PMID:22195768; http://dx.doi.org/10.1089/ten.tec.2011.056
26. Micol LA, Ananta M, Engelhardt EM, Mudera VC, Brown RA, Hubbell JA, Frey P. High-density collagen gel tubes as a matrix for primary human bladder smooth muscle cells. Biomaterials 2011; 32:1543-8; PMID:21074843; http://dx.doi.org/10.1016/j.biomaterials.2010.10.02
27. Bitar M, Salih V, Brown RA, Nazhat SN. Effect of multiple unconfined compression on cellular dense collagen scaffolds for bone tissue engineering. J Mater Sci Mater Med 2007; 18:237-44; PMID:17323154; http://dx.doi.org/10.1007/s10856-006-0685-
28. East E, Johns N, Georgiou M, Golding JP, Loughlin AJ, Kingham PJ, Phillips JB. A 3D in vitro model reveals differences in the astrocyte response elicited by potential stem cell therapies for CNS injury. Regen Med 2013; 8:739-46; PMID:24147529; http://dx.doi.org/10.2217/rme.13.6
29. Nunes SS, Miklas JW, Liu J, Aschar-Sobbi R, Xiao Y, Zhang B, Jiang J, Massé S, Gagliardi M, Hsieh A, et al. Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. Nat Methods 2013; 10:781-7; PMID:23793239; http://dx.doi.org/10.1038/nmeth.252