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The Use of Auxetic Materials in Tissue Engineering

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Abstract

A number of biological tissues have been reported as behaving in an auxetic manner, defined by a negative Poisson's ratio. This describes the deformation of tissue which expands in the axial and the transverse directions simultaneously while under uniaxial tension; and contracts axially and transversely upon uniaxial compression. The discovery of auxetic behaviour within biological tissues has implications for the recreation of the auxetic loading environment within tissue engineering. Tissue engineers strive to recreate the natural properties of biological tissue and in order to recreate the unique loading environment of cells from auxetic tissue, an auxetic scaffold is required. A number of studies have used a variety of auxetic scaffolds within tissue engineering. Investigation into the effect of auxetic micro-environments created by auxetic scaffolds on cellular behaviour has demonstrated an increased cellular proliferation and enhanced differentiation. Here, we discuss studies which have identified auxetic behaviour within biological tissues, and where cells have been cultured within auxetic scaffolds, bringing together current knowledge of the potential use of auxetic materials in tissue engineering applications and biomedical devices.

Key Words: Auxetic, Tissue Engineering, Negative Poisson’s ratio, Mechanical properties.

Introduction

Auxetic materials have unique structures which give them distinct deformation characteristics [1]. Materials deform in different ways when they have forces imparted upon them, and the vast majority behave in a conventional manner, where an object expands axially under tension while contracting in the transverse direction (Figure 1a). The negative of the ratio of transverse strain to axial strain gives a measure of how a material deforms under load which is known as the Poisson's ratio [2]. A material which expands axially but contracts transversely in response to a tensile force (Figure 1a), or expands transversely while contracting axially under compressive force (Figure 1c) has a positive Poisson's ratio. Material that displays auxetic behaviour, on the other hand, expands in the axial and one or more orthogonal directions under tension and has a negative Poisson's ratio [2] (Figure 1b). Under compressive forces auxetic materials contract both axially and transversely (Figure 1d).

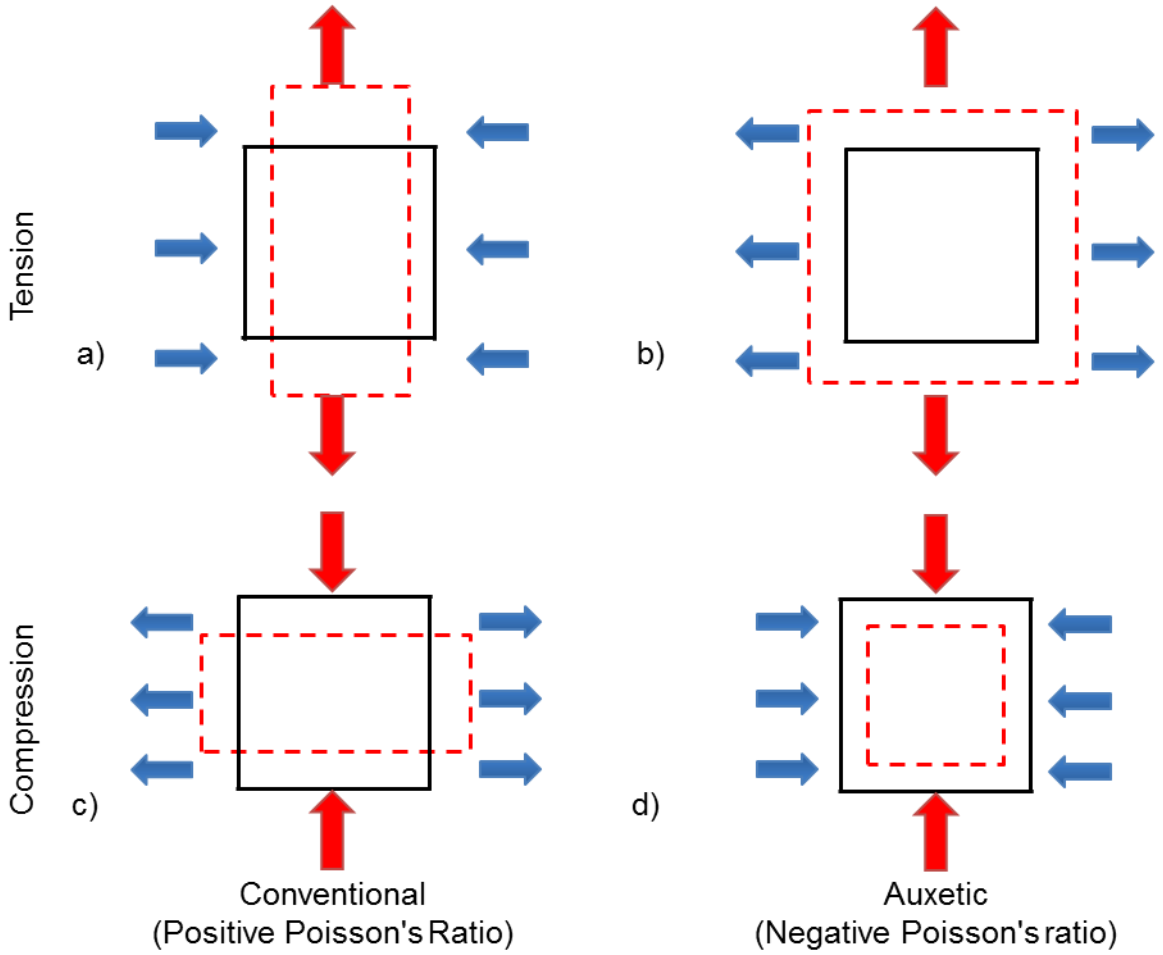

Figure 1. *Diagrammatic representation of conventional and auxetic behaviour under tensile and compressive forces. (a) Imparting an axial tensile force (red arrows) on a*

conventional material causes the deformation of the original sample (solid line) to extend axially and contract in the transverse direction (blue arrows). (b) Auxetic materials expand in both the axial and transverse directions under the same tensile force. (c) Compression of conventional materials causes axial contraction and transverse expansion. (d) Auxetic materials contract in both the axial and transverse direction under the same compressive force.

The Poisson's ratio of a material is calculated using equation 1a, b and c:

$$a)\ \varepsilon_y = \frac{\Delta y}{y_0} \qquad b)\ \varepsilon_x = \frac{\Delta x}{x_0} \qquad c)\ v_{yx} = -\left(\frac{\varepsilon_x}{\varepsilon_y}\right)$$

Equation 1: Equations for calculating axial strain (ε_y) (a), calculating transverse strain (ε_x) (b) and calculating Poisson's ratio (v_{yx}) (c). Δy = change in length, y_0 = original length, Δx = change in width, x_0 = original width.

Auxetic Biological Tissue

Naturally occurring auxetic behaviour has been found in a number of biological tissues such as cat skin [3], cancellous bone [4] and cow teat skin [5] (Figure 2 & Table 1). Since these original studies auxetic behaviour has also been reported in embryonic epithelial tissue [6, 7], arteries [8], tendons [9] and the annulus fibrosus of the intervertebral disc [10].

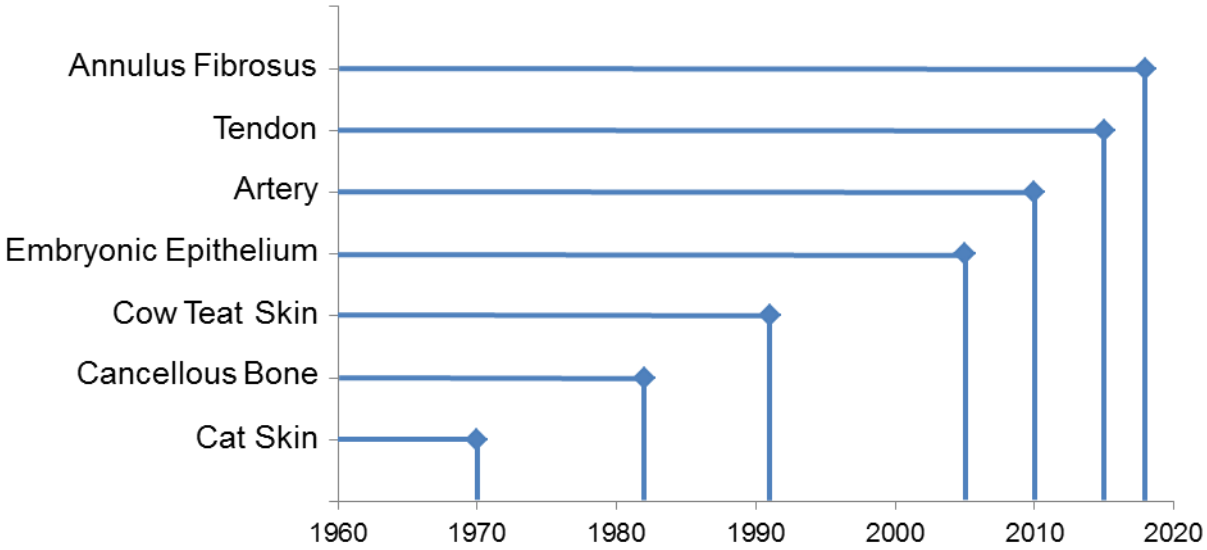

Figure 2. *Timeline of discovery of auxetic properties within biological tissues.*

Auxetic behaviour has also been reported in the nuclei of embryonic stem cells in the transition period when exiting pluripotency [11].

Year	Author	Biological Tissue	in/ex vivo	Type	Species	n Number	Technique
1970	Veronda [3]	Skin	*ex vivo*	Skin	Cat	9	Uniaxial testing and FEA
1982	Williams [4]	Bone	*ex vivo*	Tibia	Human	21	Uniaxial testing and FEA
1991	Lees *et al* [5]	Skin	*ex vivo*	Teat	Cow	6	Uniaxial testing
2005	Wiebe & Brodland [6]	Embryonic epithelia	*ex vivo*	Neuro-epithelium	Axoloti	35	Uniaxial testing
2010	Timmins *et al* [8]	Artery	*ex vivo*	Carotid Artery	Cow	4	Uniaxial testing
2015	Gatt *et al* [9]	Tendon	*ex vivo*	Achilles	Cadaveric Human	2	Uniaxial testing
			ex vivo	Peroneus Brevis	Cadaveric Human	2	Uniaxial testing
			ex vivo	Deep flexor	Pig	5	Uniaxial testing
			ex vivo	Deep flexor	Sheep	5	Uniaxial testing
			in vivo	Achilles	Human	2	MRI measurement
2018	Derrouiche *et al* [10]	Intervertebral disc	*ex vivo*	Annulus Fibrosus	Cow	45	Uniaxial Testing
2020	Dusfour *et al* [12]	Intervertebral disc	*ex vivo*	Annulus Fibrosus	Pig	20	Uniaxial Testing

Table 1. *Auxetic biological tissue reported showing: biological tissue, origin, species, n number and testing technique (FEA - finite element analysis).*

Two studies, to date have investigated the mechanical characterisation of different types of skin (Table 1) [3, 5]. Auxeticity was determined in cat skin from experimental data and validated with strain energy function finite deformation analysis [3]. Similarly a second study showed that cow teat skin was auxetic [5]. However the negative Poisson's ratio in this study was only found in the samples with a low aspect ratio (ratio of length to width) between 1.4 and 2.46. Samples outside of this range with lower (1.28) and higher (6.5-10.0) aspect ratios had positive Poisson's ratios indicating that the aspect ratio is important when determining the Poisson's ratio [5]. The authors concluded that auxetic behaviour was largely due to the skin unfolding from its corrugated form and the anisotropic fibrous network of the tissue which is similar to that of the knitted fabric, to which it was compared [5]. The data indicates that the tissue has a negative Poisson's ratio up to a certain strain, at which point the

tissue gains conventional behaviour, thus displaying a positive Poisson's ratio. This was also found to be the case for the knitted fabric that was also tested [5].

In a combined experimental and finite element analysis investigation, Williams and Lewis [4] also reported cancellous bone from the proximal tibial epiphysis was auxetic. Cancellous bone is porous, with ribs surrounding spaces forming a honeycomb like cellular structure with connecting struts aligned substantially along one of the transverse directions. This enables cancellous bone to deform in a similar way to that described for other auxetic structures [4].

Likewise Timmins *et al* [8] investigated the collagen and elastin orientation throughout the thickness of bovine arteries. They identified structural inhomogeneity in the alignment of these fibres at different depths from the luminal surface. Application of a 10% strain in the circumferential direction led to a none statistically significant tendency towards thickening of the sub-endothelial region and therefore auxetic behaviour. Concluding, Timmins *et al* [8] stated that the multi-layered inhomogeneity of fibres enables parts of the artery to deform differently. This means that artery tissue is anisotropic enabling variable deformation dependent upon loading directions and auxetic properties are possible in certain directions.

Auxetic behaviour of tendons was discovered both *ex vivo* and *in vivo* (Table 1). *Ex vivo* experiments were carried out on human Achilles tendons, human Peroneus brevis, porcine and ovine deep flexor tendons, all of which were shown to display auxetic properties when exposed to physiological strains (2%) [9]. The auxeticity of human Achilles tendon was also confirmed *in vivo* using magnetic resonance imaging [9]. The tendons tested only displayed in plane auxeticity. Gatt *et al* concluded that the crimped structure of the tendon may be responsible for the negative Poisson's ratio [9]. The normal physiological strains exerted on Achilles tendons are up to 8%, above which macroscopic rupture occurs [13]. The tendons were not further stressed to determine if the auxetic effect persisted above 2% strain.

The annulus fibrosus of the intervertebral disc has a highly organised structure with oriented collagen fibres contained within an extracellular matrix (ECM) in a lamellar configuration (Figure 3). Auxetic response has been measured experimentally in

recent studies [10, 12] and has been found to be dependent upon the strain rate and osmolarity of the local environment. This is in agreement with a chemo-mechanical model [10] based on the osmotic interaction of the negatively-charged ECM with positive ions in the surrounding physiological fluid environment. In this model, the auxetic effect arises due to the transport of fluid through the layered tissue and is mediated by stress-induced changes to the microstructure of interlamellar zones comprising ECM in the absence of oriented collagen fibres. In an alternative or complementary interpretation, the auxetic mechanism arises due to the oriented fibre-ECM lamellar structure, without the need for interlamellar zones. This purely mechanical mechanism is analogous to auxetic response known in fibre-reinforced composite laminates having similar fibre orientations to those found in the annulus fibrosus [14-16] and auxeticity has been predicted in a Finite Element Model specifically of the oriented collagen fibre-reinforced matrix of the annulus fibrosus [17].

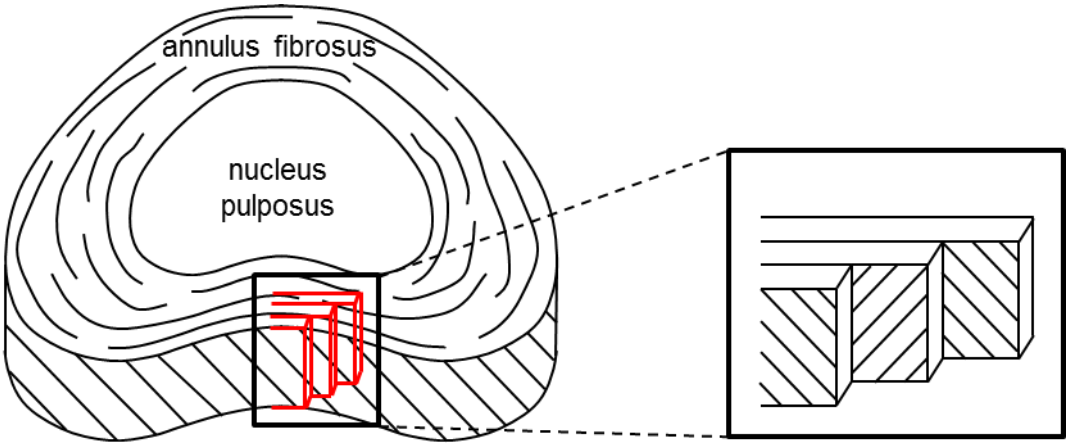

Figure 3. *Schematic diagram of the macroscopic structure of an intervertebral disc showing the different regions of nucleus pulposus and annulus fibrosus, with inset displaying a cross sectional portion of annulus fibrosus with lamellar structure and alternating directionality of collagen fibres.*

Auxeticity can also be seen in single layers of tissues, such as the epithelia. Wiebe and Brodland [6] tested the tensile axial properties of epithelium of axolotl embryos at various stages of development to 25% strain. The experimental data was used to verify a finite element model of auxeticity in neuroepithelium [7]. They determined that epithelial thickness was determined by the mechanical environment and loading

conditions within which the epithelia developed and different regions of the same epithelia have different Poisson's ratios[7].

In summary, these studies have found auxeticity in a variety of biological tissues, all of which are determined by the structure of the tissue. The effect in skin [5] and tendon [9] was postulated to be due to unfolding from its corrugated/crimped state. The auxetic effect in the sub-endothelial portion of the artery was attributed to the variable and inhomogeneous fibre alignment of elastin and collagen [8]. The auxetic effect in the annulus fibrosus has been suggested to arise from the mechanical action of oriented collagen fibres in lamellae and/or from the chemo-mechanical response of the ECM in interlamellar zones and the surrounding physiological fluid environment [10].
However, in each case, auxeticity was restricted to certain planes and strain ranges. It is possible that other biological tissues which expand in both directions under tensile load may exhibit auxeticity; whereby upon loading in one plane they also expand in another. Naturally occurring auxeticity within biological tissues provides a number of potential advantages for biological function. Thus recapitulating this property may have implications within the field of tissue engineering.

Significance of Auxetic Materials in Tissue Engineering

The foundation of tissue engineering is to create artificial tissue that mimics the natural tissue and could be used as an implant to either augment or replace biological tissues during reconstructive surgery. There are a multitude of factors and challenges that need to be considered for successful tissue engineering.

Material selection is one of the many considerations to be taken into account when fabricating auxetic tissue engineering scaffolds. Only synthetic materials have been previously used in the fabrication of auxetic scaffolds [18-27] due to the difficulty of manipulating natural materials such as ECM. The cyto-compatability and cellular adhesion should be considered and has been demonstrated in all studies developing auxetic scaffolds [18-27]. For eventual implantation applications the biocompatibility and immunogenicity of scaffolds need to tested and confirmed to ensure there are no adverse effects upon implantation. Another consideration is the physical properties

of the original tissue and the various loading conditions imparted upon it. The mechanical characteristics of engineered tissue ideally should match or perhaps enhance the mechanical properties of healthy normal host tissues, permitting full functionality, enabling it to fulfil its role *in vivo*. Cells exist in their natural *in vivo* environment embedded within extracellular matrix which is the natural scaffold of the body produced by the cells within tissues. Therefore if the target tissue is auxetic, an auxetic scaffold would most closely match the properties of this tissue. The matching of this characteristic would be beneficial in recreating the loading environment that cells would naturally experience. The phenotype of cells is altered depending upon the environmental and physical cues which are experienced, thus recapitulation of the *in vivo* environment is most likely to support normal cell phenotype [28, 29] Degradation rate of scaffolds when cultured under dynamic loading conditions is also important but is ultimately complex. Scaffolds undergo various deformation stresses and degrade at different rates when under load. The ideal scaffold degradation rate would be matched by the rate of ECM deposition to maintain the integrity of the scaffold as it breaks down, eventually being replaced with the naturally produced ECM scaffold.

In Vitro **Cultures in Auxetic Scaffolds**

A variety of auxetic constructs have been investigated for use as tissue engineering scaffolds (Table 2). A number of techniques have been employed using synthetic materials to fabricate auxetic scaffolds to grow cells within a 3D environment (Figures 4 & 5).

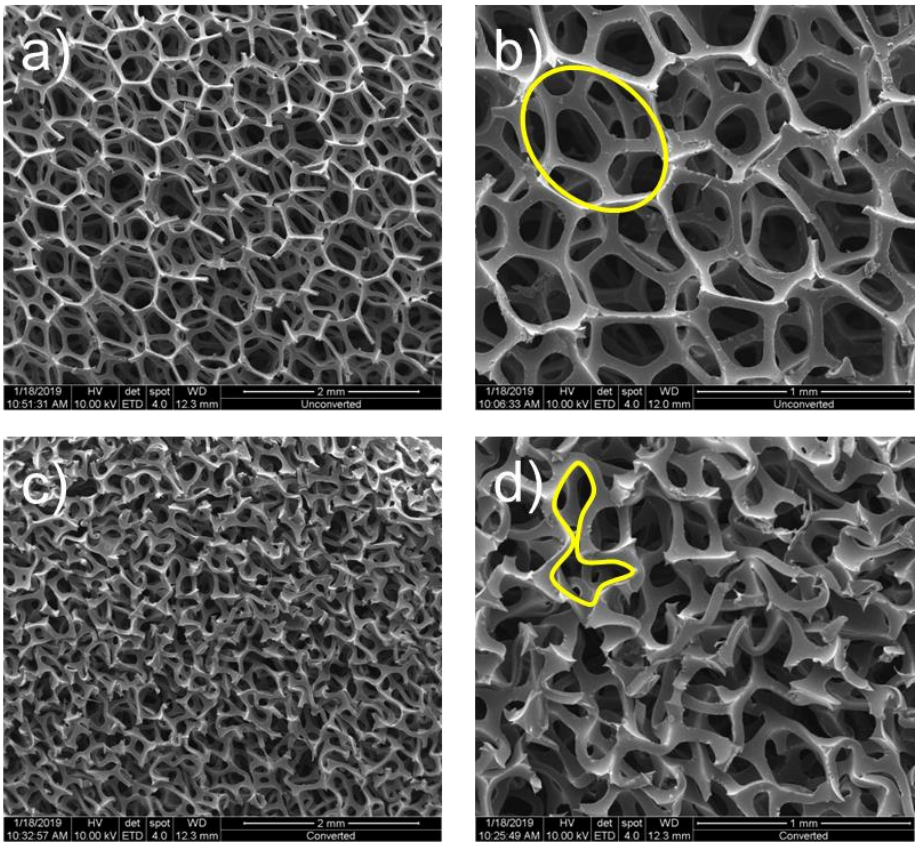

Figure 4, *SEM images of cross sections of uncompressed and tri-axially compressed polyurethane foam. a) Uncompressed foam showing pores with regular well-ordered structure (scale bar 2mm). b) Higher magnification image of uncompressed foam with example uniform pore highlighted in yellow (scale bar 1mm). c) tri-axially compressed polyurethane foam showing irregular pores with a re-entrant structure (scale bar 2mm). d) Higher magnification image of foam with example re-entrant bowtie structure highlighted in yellow (scale bar 1mm).*

Tri-axial compression has been employed to create auxetic scaffolds from polyurethane [20, 25, 26] and poly(lactic-co-glycolic acid) (PLGA)[22, 23] with similar results to the triaxial compression of polyurethane foam (Figure 4). Alternatively the precision of 3D printing/digital mirror device stereolithography to create auxetic geometries to create auxetic scaffolds has also been employed using a range of auxetic geometries [18, 19, 21, 24, 27] (Figure 5).

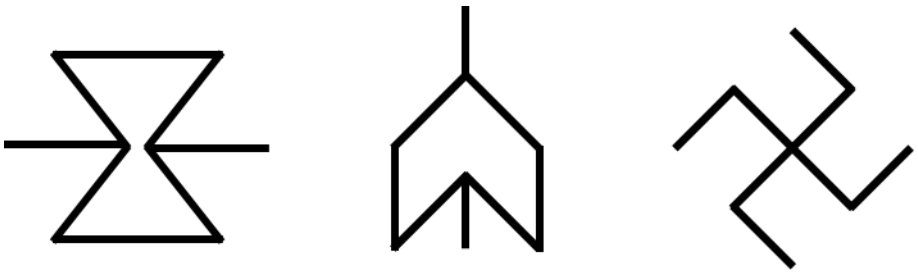

Figure 5. *Geometries used to create auxetic scaffolds using 3D printing techniques.*

Cyto-compatability has been confirmed on such scaffolds by culturing a number of cell lines on the scaffolds (Table 2). For example, Sonam *et al* [18, 19] cultured human mesenchymal stromal cells (hMSC) on Poly(ethylene glycol) diacrylate (PEGDA) scaffolds.

Whilst Yan *et al* [25] showed that there was an increase in cell proliferation in both human induced pluripotent stem cells (iPSCs) and mouse embryonic stem cells (ES-D3) when cultured on negative Poisson's ratio scaffolds. Similarly, Lee *et al* [24] identified the same increased proliferation in hMSCs when cultured on scaffolds with a negative Poisson's ratio.

Yan *et al* [25] also reported an increase in cellular differentiation toward a neural lineage in both human iPSCs and murine ES-D3 cells. This increased differentiating effect of a negative Poisson's ratio scaffold was also found in human iPSCs and murine ES-D3 cells but down a vascular lineage [26]. These studies indicate that auxetic scaffolds influence cellular differentiation and cellular phenotype.

Several studies also investigated the effect of auxetic scaffolds under a compressive load on human osteoblasts [22, 23] and porcine primary chondrocytes [20]. Demonstrating compressive load increased the proliferation of cells.

Auxetic scaffolds as well as supporting the growth of a variety of cell types, also have beneficial effects (Table 2). Including increased cellular proliferation and an increase in differentiation of embryonic stem cells down vascular and neural lineages [25, 26], and thus could be useful in tissue engineering.

Year	Author	Scaffold Material	Pore size (μm)	Cell Type	Species	Phenotype/origin	Static/ Dynamic	Load	Strain Range	Time Loaded	Effect
2012	Sonam *et al* [19]	PEGDA		HMSC	Human	bone marrow	Static	None	N/A	N/A	Grow on scaffold
2012	Sonam *et al* [18]	PEGDA		HMSC	Human	bone marrow	Static	None	N/A	N/A	Grow on scaffold
2013	Park & Kim [20]	Polyurethane		Chondrocytes	Porcine	primary from cartilage	Static	Compression 0.3N	20%	constant	Increased proliferation
2013	Zhang *et al* [21]	PEGDA	≤ 1	10T1/2	Mouse	embyronic fibroblast	Static	None	N/A	N/A	Unable to divide
2016	Choi *et al* [22]	PLGA	355-400	MG-63	Human	osteoblast like	Static	Compression 19.6N	10%	constant	Increased proliferation
2016	Choi *et al* [23]	HA/PLGA	355-400	MG-63	Human	osteoblast like	Dynamic	Compression 19.6N	15%	4 hours per day @ 0/5/15 min cycles	Increased proliferation
2016	Lee *et al* [24]	PEGDA		hTMSC	Human	turbanate	Static	None	N/A	N/A	More cells on NPR scaffold
2017	Yan *et al* [25]	Polyurethane	250-300	ES-D3	Mouse	embryonic multipotent stem cell (blastocyst)	Static	None	N/A	N/A	Increased proliferation and neural differentiation
				iPSK3	Human	foreskin fibroblast	Static	None	N/A	N/A	Increased proliferation and neural differentiation
2017	Song *et al* [26]	Polyurethane and polyester	250-300	ES-D3	Mouse	embryonic multipotent stem cell (blastocyst)	Static	None	N/A	N/A	Increased vascular differentiation
				iPSK3	Human	foreskin fibroblast	Static	None	N/A	N/A	Increased vascular differentiation
2017	Warner *et al* [27]	Polyurethane	Varied	C3H/10T1/2	Mouse	embryonic fibroblast	Static	None	N/A	N/A	Grow on scaffold
				C3H/C2C12	Mouse	myoblast	Static	None	N/A	N/A	Grow on scaffold

Table 2. *Auxetic scaffold studies showing scaffold material, pore size, cell type, species, phenotype, loading conditions and effect.*

Micrometer-Scale Auxetic Scaffolds

Zhang *et al* [21] developed a method to fabricate suspended web structures with ≤1μm pores with negative Poisson's ratio geometry, meaning that cells are larger than the scaffold pores and therefore could attach to more than one rib of the scaffold. Thus, this scaffold could in principle enable the investigation of the true effect of a negative Poisson's ratio on the cells. However, it was observed that the forces of cells on the scaffold caused it to deform, rather than the mechanical properties of the scaffold itself influencing cell growth. It was concluded that the scaffold ribs were too small and there was not enough resistance to impart such a property from the auxetic scaffold on the cells. Furthermore the lack of scaffold support resulted in the cells being unable to divide normally [21].

Verifying Cyto-Compatibility of Auxetic Scaffolds

A number of studies have cultured cells on auxetic scaffolds to determine whether cells attach, and to check cyto-compatibility [18, 19, 24, 27].

Human MSCs were cultured on zero Poisson's ratio scaffolds [18] and positive and negative Poisson's ratio constructs [19] for 7 days before being stained with phalloidin (actin) and diamidino-2-phenylindole (DAPI) (nuclei). This showed that MSCs could be maintained on the scaffolds for up to a week. Similarly Lee *et al* [24] and Warner *et al* [27] seeded Human MSCs, embryonic fibroblasts and myoblast cells onto scaffolds to ensure cellular attachment and survival, culturing cells for 11 and 12 days respectively. These studies demonstrated the short-term culture of cells on auxetic scaffolds was possible. However no further investigation into cellular behaviour was attempted.

Cellular Differentiation of Stem Cells on Auxetic Scaffolds

Two studies to date have investigated the effect of auxetic scaffolds on the differentiation of stem cells [25, 26].

Neural Differentiation

Yan *et al* [25] set out to investigate the effect of the Poisson's ratio of polyurethane scaffolds on human and mouse stem cell differentiation. Stem cells cultured within auxetic scaffolds formed aggregates that were smaller than aggregates formed in

conventional scaffolds. It was found that the metabolic activity of the cells harvested from the scaffolds was significantly higher within the auxetic scaffolds than within the conventional scaffolds. Further investigations discovered that smaller aggregates showed a higher metabolic activity due to formation of more compact aggregates within the auxetic scaffolds associated with strong cytoskeletal architecture. When cultures were treated with an actin depolymerisation agent, the aggregates within the auxetic scaffolds were considerably larger due to the depolymerisation of the actin. This effect was not seen in the aggregates that were cultured in conventional scaffolds which were found to remain unchanged in size [25]. These findings suggest that the auxetic structure of the scaffold creates a bio-physical environment which affected the organisation of actin.

However, within this study no load was applied to the scaffolds, so the biophysical environment was created by the properties of the scaffold alone. The auxetic scaffolds themselves did not cause the stem cells to differentiate down a neural lineage. The scaffolds did however enhance the differentiation of cells when cultured under neural induction conditions [25].

Vascular Differentiation

Song *et al* [26] investigated the effect of auxetic polyurethane scaffolds on vascular differentiation of pluripotent stem cells. Mouse embryonic stem cells were found to be significantly more proliferative within the auxetic scaffold following 3 days of culture. However after day 3, this effect was no longer evident. There was also a downregulation of stem like markers (Oct-4 and Nanog) in the cells cultured in the auxetic scaffold when compared to the regular scaffold, demonstrating that cells within the auxetic scaffolds were differentiating. Furthermore a significant increase in vascular markers: CD31 and VE-cadherin was observed following 11 days. There was also an alteration in ECM production (vitronectin and laminin) demonstrating that this may also be affected by the biophysical environment created by the auxetic scaffolds [26].

Human iPSCs showed the same level of proliferation when cultured on both auxetic and regular scaffolds. Although levels of proliferation were the same, there was higher expression of vascular markers (CD31 and VE- cadherin) in the cells from the

auxetic scaffolds than the regular scaffolds. This shows the differentiating effect of auxetic scaffolds was seen in both hiPSCs and mouse embryonic stem cells [26]. Similar to Yan *et al* [25] the culture of cells on auxetic scaffolds was carried out in the absence of load and therefore the differentiating effect was due to an unloaded auxetic scaffold.

All studies that have been discussed thus far have been cultured within auxetic scaffolds in an unloaded passive state. Tissues are very rarely static, but are dynamic and are loaded in multiple axes. In order to recapitulate the natural environment of tissues, scaffolds need to be cultured dynamically under load.

Auxetic Scaffolds Under Compressive Load *In Vitro*

The following studies have investigated cell behaviour in a loaded environment.

Auxetic Scaffolds in Cartilage Tissue Engineering.

Park and Kim [20] fabricated auxetic scaffolds by thermomechanical tri-axial compression of conventional parent polyurethane foam. Generating scaffolds which maintained a negative Poisson's ratio of -0.4 ±0.12 at 20% compressive strain. Primary chondrocytes were isolated from the lateral and medial condyle of pig femurs, and cultured on the auxetic scaffolds under static compression (20%) for a period of 5 days. Chondrocyte proliferation was increased at all time points (1, 3 and 5 days) within the auxetic scaffolds, when compared to the unconverted control, and was significant after 3 days but not after 5 days. Furthermore the synthesis of collagen was also increased in auxetic scaffolds compared to conventional scaffolds at 3 and 5 days.

The authors postulate that the lack of significance in the proliferation of chondrocytes between 3 and 5 days could be due to the stress relaxation and the viscoelastic properties of the polyurethane scaffold [20]. The study however did not address this, and assessing the mechanical properties of the scaffolds in this way warrants further investigation.

Auxetic Scaffolds in Bone Tissue Engineering.

Choi *et al* [22] fabricated auxetic poly(lactide-co-glycolide) (PLGA) scaffolds using a solvent casting/salt leaching technique. Auxeticity was induced by thermomechanical tri-axial compression followed by cooling of the scaffolds to room temperature within the moulds. Auxeticity was measured and confirmed using dry samples. The compressive strength of the scaffolds was reduced significantly when wet and was further reduced in physiological conditions using Dulbecco's modified eagle's medium at 37°C. Scaffolds were incubated in phosphate buffered saline at 37°C on a shaking incubator and the degradation of the scaffold was measured by the percentage weight loss and changes in morphology. Although the scaffolds had lost 17% of total weight, the scaffolds collapsed after 5 weeks, suggesting that the integrity of the scaffold was completely compromised and the deformation characteristics would be very different from the original state.

Choi *et al* used this scaffold together with conventional non-auxetic control scaffolds to culture osteoblast-like MG-63 cells. The proliferation of these cells was increased following 1 day of culture with a constant compressive force of 19.6N (10% strain) when cultured in non-auxetic scaffolds and compared to a scaffold with no load applied. The proliferation was further increased (1.46 times) when cultured within the auxetic scaffold under the same loading conditions (19.6N – 10% strain). However, after 3 days in culture, whilst proliferation of MG-63 cells was significantly increased in loaded scaffolds, no difference was seen between auxetic and non-auxetic scaffolds under compression, indicating that it was the compressive force that was having this effect rather than the auxeticity of the scaffold. After 5 days this effect was dissipated and there was no further significant increase in cell proliferation [22].

This study focussed on cellular proliferation and total cell number rather than cellular phenotype or differentiation. If the cells were merely more active, this could mean that the cells are not necessarily more proliferative even though the results suggest that this is the case. This is something that requires consideration when determining whether an auxetic environment increases proliferation.

Unfortunately the auxetic properties of scaffolds in this study were only determined using dry samples, which were shown to be only marginally auxetic, with the lowest

value of Poisson's ratio being -0.07. The scaffolds exist in culture in a wet state and it would therefore be more pertinent to determine whether the scaffolds were auxetic when wet. The compressive strength of the scaffold was significantly reduced when wet, and therefore the scaffold had altered mechanical properties to those measured when dry. Thus in culture, whether or not the scaffolds retain a negative Poisson's ratio and the benefits associated with auxetic properties [23], remains unclear.

The differences observed in cell proliferation at day 3, could be attributed to the compressive force rather than the auxeticity of the scaffold as there is no significant difference between the auxetic and non-auxetic scaffolds under compression, with the only significant differences being between the unloaded and loaded scaffolds. This could be due to the cell type, as osteoblasts are well known to increase activity under load, increasing proliferation rates [30] and osteogenic activity [31]. The proliferative effect of auxetic scaffolds under compression within this study is very short with the only significant difference between scaffolds being seen after 1 day [22].

Further investigations by Choi *et al* [22] fabricated composite auxetic scaffolds from PLGA and hydroxyapatite using the same technique, and were compared to auxetic PLGA scaffolds alone. The study demonstrated the inclusion of hydroxyapatite in a composite PLGA/hydroxyapatite scaffold increased the mechanical properties under compressive strain when compared to a PLGA scaffold. The mechanical properties of the scaffolds were reduced by 70% in the wet state. Recovery of the scaffolds to their original dimensions after compressive force was applied was also attenuated in the hydrated/wet state after 5 minutes. All PLGA/hydroxyapatite scaffolds recovered more than the PLGA scaffold in agreement with the addition of hydroxyapatite increasing the mechanical properties.

Furthermore, MG-63 cells exhibited increased levels of proliferation on PLGA/ hydroxyapatite scaffolds compared to PLGA scaffolds when no load was exerted. The use of cyclic compressive strain further increased the proliferation of MG-63 cells on PLGA/hydroxyapatite scaffolds. This was in agreement with Kaspar *et al* [30] who showed that osteoblast proliferation was increased by cyclic uniaxial loading.
The auxeticity here was once again only determined from dry samples. This again raises the question whether scaffolds would still exhibit auxeticity in a hydrated form.

Scaffolds would exist within the body at physiological conditions and therefore auxeticity should be determined within the hydrated state to determine if it is in fact the auxeticity that is imparting the effects seen, rather than an effect of compressive load.

No histological analysis within either this or the previous study raises the question of whether the cells are attached or associated with the scaffolds; or are just suspended within the pores. This is essential in determining whether cells are experiencing the true forces from the scaffolds and whether the auxetic property is contributing to the effects on cell proliferation.

These three studies of auxetic scaffolds under compressive load demonstrate increases in the proliferation of osteoblasts and chondrocytes [20, 22, 23]. However all studies were only performed over 5 days, and longer studies would show whether these effects could be regained or maintained over a longer period of time.

To date, no studies investigating the effect of tensile forces upon cells grown within auxetic scaffolds have been reported, although there are studies where cells are cultured on auxetic scaffolds without load [18, 19, 21, 24-27].

Auxetic Biomedical Devices

The exploitation of auxetic materials and their unique deformation characteristics has been explored within a number of biomedical applications.

Burriesci and Bergamasco [32], published a patent on annuloplasty prostheses with an auxetic structure. Annuloplasty is a surgical procedure to repair a damaged heart valve to ensure that blood flow is unidirectional. It involves the implantation of a closed or open ring structure on the annulus of the valve to enable it to recover its physiological shape and therefore its function. An auxetic prosthesis is advantageous as it is flexible and can be moulded to fit to the physiological shape of the annulus without crimping [33]. Under deformation the auxetic behaviour of the prosthesis acts to stabilise the annulus. During use, the prosthesis undergoes loading in various directions at once and the stabilising capacity of the auxetic prosthesis would be beneficial [32].

Martz *et al* [34] fabricated an artificial intervertebral disc made from high density polyethylene, made by drilling holes to create an auxetic honeycomb structure through the core of the disc. Intervertebral discs are load bearing and therefore are under a number of mechanical strains including compressive, tensile (in the outer region of the disc) and torsion forces. Intervertebral discs can fail leading to disc protrusion or herniation which leads to nerve impingement causing pain. The auxetic behaviour of this device which shrinks inwards under compression is proposed to prevent the bulging of the disc and hence decrease the chance of nerve impingement [34]. Another patent for an auxetic prosthetic intervertebral disc implant has also been granted [35].

The use of auxetic materials has also been explored within hip prostheses due to the enhanced strain distribution produced [36] and patents have been filed [37]. Strain distribution is being further investigated within meta-implants which have auxetic and conventional components [38, 39]

Auxetic structures have also been employed in the creation of oesophageal stents for potential palliative treatment of oesophageal cancer [40]. Thus auxetic materials show potential for use within a number of applications.

Future Outlook for Auxetics in Biomedical Applications

More investigations will give further understanding of the effects of the loaded auxetic micro environment upon cells. These may elucidate other beneficial effects which can be used in future cultures of cells within tissue engineering of auxetic tissue. Advances in manufacturing technologies and fabrication techniques will also enable their application in the future development of further auxetic scaffolds, and could enable closer control over mechanical properties matching these to the natural tissues.

The presence of natural auxeticity within embryological epithelium provides new avenues for embryological research where the influence of such properties on embryological formation could be investigated. Furthermore, evidence is emerging that could link auxeticity to disease. Two studies have recently demonstrated auxetic behaviour within the annulus fibrosus[10, 12]. Its laminar structure is made up of ECM

proteins such as collagens that make up the natural scaffold of the IVD. In IVD degeneration there is a loss of ECM which coincides with alterations in mechanical properties[41]. Alterations in the types, amounts and organisation of collagens within this tissue may lead to a loss of auxeticity during degeneration which could explain at least in part Annular tear formation during daily loading. Hence a reasonable hypothesis is a reduction of ECM causes loss of auxeticity and mechanical properties and therefore is important in disease pathogenesis. Emerging research has the potential to elucidate further links between auxetic properties of tissues and disease.

Conclusion

A number of biological tissues have been shown to display auxetic properties leading to an interest in the use of auxetic materials within tissue engineering. It is likely further reports of auxetic biological tissues will appear in due course, once the significance of the effect becomes apparent. An increasing number of investigations into the culture of cells within auxetic scaffolds are being reported with varying degrees of depth. While some studies merely culture cells within auxetic scaffolds for a week to determine cellular attachment and cyto-compatability, others have investigated the effects on cellular behaviour such as proliferation and differentiation. Only three studies investigated the effect of loading on cells within auxetic scaffolds imparting a dynamic loading environment on cells which is much more physiologically relevant. Furthermore a number of biomedical devices have been proposed where auxetic properties are predicted to provide beneficial effects. Together, these studies support the potential application of auxetic materials in tissue engineering and biomedical devices.

Conflict of interest

PM, CLM, NJM declare no conflict of interest with the content of this review, AA is a named inventor on one of the patents referred to in the review.

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