Mechanical environment for *in vitro* cartilage tissue engineering assisted by *in silico* models

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ABSTRACT

Mechanobiological study of chondrogenic cells and multipotent stem cells for articular cartilage tissue engineering (CTE) has been widely explored. The mechanical stimulation in terms of wall shear stress, hydrostatic pressure and mechanical strain has been applied in CTE in vitro. It has been found that the mechanical stimulation at a certain range can accelerate the chondrogenesis and articular cartilage tissue regeneration. This review explicitly focuses on the study of the influence of the mechanical environment on proliferation and extracellular matrix production of chondrocytes in vitro for CTE. The multidisciplinary approaches used in previous studies and the need for in silico methods to be used in parallel with in vitro methods are also discussed. The information from this review is expected to direct facial CTE research, in which mechanobiology has not been widely explored yet.

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Introduction

Current optimal management in the reconstruction of facial cartilage involves the use of autograft, allograft, or xenograft chondrocyte sources, which present the difficulties of donor site morbidity, transplant rejection, and low source availability.[1] Cartilage tissue engineering (CTE) provides a promising solution, which involves the seeding of multipotent stem cells, chondrogenic progenitor cells, or chondrocytes onto porous scaffolds.[2] This method is followed by the expansion of culture volume and the generation of neo-synthesised extracellular matrix (ECM) prior to implantation.[3] Cartilage tissue growth within the scaffolds is a complex process with multiple mechanical, biochemical, and genetic factors influencing chondrogenesis, further complicated by the temporality of the process. If the cell/tissue culture time can be shortened, patients who have facial cartilage damage can receive the implants at an appropriate time. Therefore, there is a demand to accelerate the chondrogenesis as well as proliferation and ECM production of chondrocytes during cell culturing for facial CTE.

Different *in vitro* studies used different cell types, for example, some have used mesenchymal stem cells,[3, 4] and others used primary chondrocytes,[5, 6] for CTE. The majority of these studies have found that the mechanical environment can influence differentiation, proliferation, and ECM production of primary chondrocytes and mesenchymal stem cells in articular CTE experiments.[7] Mechanobiology for facial CTE has not been extensively investigated yet. The mechanical environment influences CTE in both static and dynamic culture conditions. For static culture condition, the mechanical factors that can influence the chondrocytes' behaviours are stiffness of materials used for housing the cells.[8] For dynamic culture condition, mechanical factors that influence the chondrocyte behaviours include fluid-induced wall shear stress (WSS), hydrostatic pressure and mechanical strain.[7] These mechanical stimulation processes are applied to cells using bioreactors such as perfusion bioreactors, spinner flasks and mechanical compression bioreactors. Computational studies have demonstrated that the scaffold geometric features such as porosity, pore size and pore shape also influence these mechanical stimulations of cells within three-dimensional (3D) scaffolds.[9]

Thus, one of the promising strategies for guiding cellular behaviour is to tune the scaffold geometric features and scaffold materials mechanical properties.[10] Recent advances in scaffold design and manufacturing such as 3D printing have permitted the precise control of scaffold geometric features including porosities, pore size and curvature, as well as mechanical properties such as stiffness.[11] Critically, the field of CTE has now reached a juncture, in which the combination of predefined, quantifiable mechanical forces is possible. However, optimising these parameters in conjunction to enhance the cellular behaviours (such as chondrogenic differentiation, proliferation and ECM production) presents a challenge due to the high cost associated with the trial-and-error *in vitro*/*in vivo* experiments. With the emergence of *in silico* (computational) tissue engineering, it may help reduce, refine and replace some of the *in vitro*/*in vivo* experiments. Therefore, a multidisciplinary approach is essential using *in silico* methods to determine the optimum scaffold geometric features and mechanical properties for both static and dynamic cell culture conditions; and/or bioreactor loading condition for dynamic cell culture.[12]

This review will explicitly focus on the study of the influence of mechanical environment on proliferation and matrix production of chondrocytes *in vitro* for CTE. As chondrocytes mechanobiology has been more widely investigated for articular CTE than that for facial CTE, we will discuss the literature mostly based on the articular CTE in this review. The use of *in silico* approaches to model the intricate mechanical stimulation for refining *in vitro* tissue engineering studies is also discussed. Due to the similar requirements for articular CTE and facial CTE (e.g., accelerated chondrogenesis, proliferation and ECM production), the knowledge of mechanobiology for articular CTE can be extrapolated for facial CTE studies. Consequently, future research is suggested for the facial CTE field.

Static Culturing Condition

Effect of substrate stiffness on tissue growth

Cells exert forces on the material to which they adhere, and also receive mechanical feedback from these surfaces, even under static culturing conditions.[13] Through mechanotransduction, this mechanical feedback influences the adhesion strength and cytoskeletal architecture of the cells, and thereby influences the cellular behaviours. It has been shown that the stiffness of the substrate material affects chondrocyte behaviours such as proliferation and ECM production.[14] The material stiffness is sensed via molecular pathway (such as Rac1), which modulates S-phase entry and control cell proliferation.[15, 16] Therefore, changing the substrate material stiffness will influence the modular pathway, and consequently influence cell proliferation. Also, cells grown on stiff substrates with different stiffnesses generate different amounts of F-actin, different spread, and display varying yes-associated protein/transcriptional co-activator with PDZ-binding motif in the nucleus, which in turn, affects the cell proliferation.[17] **Table 1** summarises previous *in vitro* studies that demonstrated the substrate material stiffness dependent phenomena of chondrocytes.[5, 18-23]

In silico models for tissue engineering under static condition

In this section, the *in silico* models for modelling the cell mechanobiological behaviour in tissue engineering based on the cell-material interaction are discussed. *In silico* modelling is usually used for simulating the cell behaviours on substrates in static culturing conditions (**Figure 1A**). The aim is to evaluate and optimise the material in terms of the properties (such as mechanical properties). This can help direct the *in vitro* work and avoid excessive trial-and-error for *in vitro* experiments.

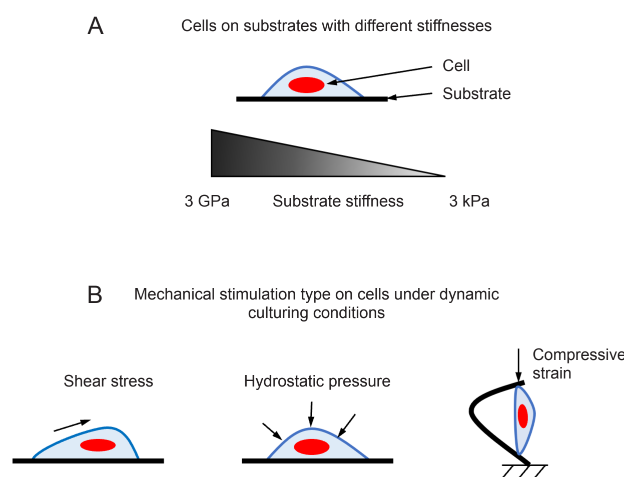

Figure 1. (A) In static culturing condition, cells are cultured on substrates with different stiffnesses. (B) Different types of mechanical stimulations have been applied to cells in dynamic culturing conditions.

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Table 1. Chondrocytes respond to substrates with different stiffness in cell culturing

Chondrocytes resource	Substrate material & stiffness	Biological responses	Reference
Porcine	Hydrogel: (i) 3.7 kPa (ii) 53 kPa	• Type I and II collagen: no difference • Cell number and ECM amount: (i) > (ii)	5
Sheep	Hydrogel: (i) 5 kPa (ii) 10 kPa (iii) 20 kPa	• Aggrecan, Col2a1, and Sox9 levels: (i) > (ii) or (iii) • Organisation of actin cytoskeleton (co-related to loss of chondrocyte phenotype): (i) < (ii) or (iii)	18
Bovine	Hydrogel: (i) 3.8 kPa (ii) 17.1 kPa (iii) 29.9 kPa	• Round cell morphology and decreased actin cytoskeletal organisation: (iii) > (ii) > (i) • sGAG/DNA, Col2a1 and aggrecan expressions: (iii) > (ii) > (i)	19
Bovine	Hydrogel: (i) 1 kPa (ii) 15 kPa (iii) 30 kPa	• sGAG and type II collagen expressions: (iii) > (ii) > (i)	20
Murine	Type II collagen-coated PAM: 0.2 – 1.1 MPa	• Proteoglycan deposition, and *Sox9*, *Col2a1*, and aggrecan mRNA expressions are greatest under the substrate stiffness of 0.5 MPa • mRNA level: no difference	21
Bovine	PAM gel coated with type I collagen: (i) 4 kPa (ii) 10 kPa (iii) 40 kPa (iv) 100 kPa	• Differentiated chondrocyte phenotype: (i) > (ii) – (iv) • Type II collagen and aggrecan genes: (i) > (ii) – (iv) • Type I collagen: (i) < (ii) – (iv)	22
Human	(i) PDMS: 4.8 MPa (ii) PS: 2.9 GPa	• Sox9 and type II collagen expressions: (i) > (ii) • Proliferation: no difference	23

Note: Col2a1: type II collagen alpha-1; ECM: extracellular matrix; PAM: polyacrylamide; PDMS: polydimethylsiloxane; PS: polystyrene; sGAG: sulphated glycosaminoglycans; SOX9: SRY-box transcription factor 9.

The mechanical interaction between the substrate material and cells is via focal adhesions that connect intracellular actin bundles and the extracellular substrate: F-actin and myosin generate and transmit forces.[24] Therefore, currently, some *in silico* models of single cell mechanics focus on modelling the force generation and transmission within sub-cellular components such as stress fibres (SFs) and focal adhesions, and the corresponding remodelling of the SFs. These *in silico* models aim to predict the cellular behaviours that are influenced by the substrate stiffness. Most of the cell mechanics models have employed a coupled thermodynamics and mechanics approach for studying single cell behaviour.[24-27] For SF, it consists of three phenomena: SF formation is triggered by an activation signal; reduction in fibre tension leads to fibre dissociation; and the contractile behaviour of SFs is similar to muscle mechanics.[24] This chemo-mechanical behaviour of SF is commonly modelled by a simplified Hill-like equation as described[28] (Equation 1):

$$\frac{\sigma}{\sigma_o} = \begin{cases} 0 & \frac{\dot{\varepsilon}}{\dot{\varepsilon_o}} < -\frac{\eta}{k_v} \\ 1 + \frac{\bar{k}_v}{\eta}\left(\frac{\dot{\varepsilon}}{\dot{\varepsilon_o}}\right) & -\frac{\eta}{k_v} \leq \frac{\dot{\varepsilon}}{\dot{\varepsilon_o}} \leq 0 \\ 1 & \frac{\dot{\varepsilon}}{\dot{\varepsilon_o}} > 0 \end{cases} \quad (1)$$

where η ($0 \leq \eta \leq 1$) is a nondimensional biochemical state parameter for characterising the recruitment of actin and myosin in an SF bundle; nondimensional constant $\bar{k}_v$ is the fractional reduction in fibre stress upon increasing the shortening rate by $\dot{\varepsilon_o}$; $\dot{\varepsilon}$ is the axial fibre strain rate.

For focal adhesion, its formation is modelled by a mechano-chemical model,[29, 30] which mathematically describes the relationship between the chemical potential, mechanical energy and integrins concentration.

Scaling up groups of cells (rather than single cell), some other *in silico* models have simulated the cellular activities that are influenced by substrate stiffness for CTE application using an agent-based modelling approach.[31, 32] These *in silico* models can be applied to tuning the 3D scaffold mechanical properties such as stiffness for CTE application.

Dynamic Culturing Condition

To study the cell physiology and pathology, mechanical stimulation needs to be applied to cells in CTE experiments *in vitro* with dynamic culturing conditions. The methods of mechanical stimulation that can influence chondrocyte growth are dynamic compression, shear forces, and hydrostatic forces as illustrated in **Figure 1B**. Dynamic culturing conditions have the potential to support higher cell populations than static conditions,[33] induce chondrogenic differentiation of mesenchymal stem cells, and enhance the production of the cartilage-specific ECM.[34] The mechanical stimulation is considered as a driving factor for regulating the calcium ions (Ca^{2+}) entry, primarily through voltage-operated calcium channels, transient receptor potential channels, and purinergic receptors.[35] In addition to the growth factors, such as: transforming growth factor-β, insulin-like growth factor, and bone morphogenetic protein-2, -4, -7, Ca^{2+} is needed to

regulate the cell functions (e.g., synthesis of ECM components) during chondrogenic process.[35, 36] In the following subsections, different types of mechanical stimulation for CTE are discussed.

Effect of dynamic compression on cartilaginous tissue growth

To replicate the *in vivo* environment of cyclical compressive loading induced by physical activity (such as walking or running), dynamic compression in the form of uniaxial cyclical loading force, has been applied to the tissue culture with a specific and quantifiable frequency, amplitude, and total duration.[37-39]

In many *in vitro* CTE studies, mechanical stimulation has been applied to cell-laden hydrogels to stimulate cells. Many studies have found that continuous compressive loading yields superior biological and mechanical properties of constructs. For instance, Nebelung et al.[37] utilised human osteoarthritic chondrocytes that were seeded into type I collagen hydrogels at a density of 2×10^5 cells/mL in a compression bioreactor system applying a compressive stimulation of 10% loading at a frequency of 0.3 Hz for 28 days. They found that type II collagen and proteoglycan production was significantly increased relative to unstimulated controls, as well as gene expression of type I collagen, type II collagen and matrix metalloproteinase-13. Mechanically stimulated constructs demonstrated higher values of elastic stiffness.[37] This phenomenon was also observed by Sawatjui et al.[40] who used scaffolds made from silk fibroin with gelatine/chondroitin sulfate/hyaluronate in a ratio of 2:1 for culturing articular chondrocytes under dynamic (10% strain, 1 Hz, for 1 hour/d) and static compression for 2 weeks. In addition to upregulated biological expression including type II collagen and aggrecan under dynamic condition, the resultant stiffness of tissue-scaffold struts was higher under dynamic condition.[40] Another study by Grogan et al.[41] sought to establish if there was a potentiating effect of perfusion and dynamic compression in the chondrogenicity of articular chondrocytes embedded in 2% alginate. Constructs were placed in a control static culture, a bioreactor for perfusion alone (at 100 mL/min) and a bioreactor for perfusion and dynamic compression (of 20% strain at 0.5 Hz, for 1 hour each day). *Col2a1* mRNA expression levels were significantly raised in both bioreactors relative to the control at days 7 and 14. However, there were no significant differences in gene expression between the two bioreactor protocols at day 7 or 14, suggesting that dynamic compression did not further promote chondrogenicity relative to perfusion alone.[41]

Some other studies used porous biomaterial scaffolds for housing the cells in CTE *in vitro* experiments. For example, Sawatjui et al.[40] assessed the response of healthy human articular chondrocytes seeded on 3D PEGT/PBT constructs to dynamic compression (5% strain at 0.1 Hz for 6 cycles of 2 hours duration over 3 days). It was found that glycosaminoglycan (GAG) synthesis, accumulation, and release all significantly increased with dynamic compression relative to control scaffolds, but this increase was heavily dependent on the baseline GAG/DNA ratio of the samples.[40] This suggested that the donor tissue characteristics before mechanical stimulation were important factors in determining response.

Some recent studies applied mechanical compression loading to the cartilage explant in bioreactors.[39, 42, 43] Engstrøm et al.[39] found that compressive loading alters cartilage tissue turnover and enforces the need to include mechanical loading in a translation *ex vivo* cartilage model. In another study, a bone-cartilage explant was investigated under the physiologically-relevant compression.[43] It was found that the physiological loading rapidly activated markers of ECM synthesis and tissue homeostasis via the anabolic transforming growth factor-β/Smad3 pathway, whereas supra-physiological compression induces an initial remodelling response for inhibiting tissue degeneration.[43]

Effect of wall shear stress on cartilaginous tissue growth

WSS is a force tangential to the cell wall. It can be induced by direct solid-on-solid stimulation (contact shear) or through hydrodynamic forces (fluid shear). Contact shear is present physiologically as cartilage rubs against cartilage with the rotating movements of joints. This can be recreated *in vitro* using bioreactors, which maintain pre-determined contact shear frequencies and amplitudes. Fluid-induced shear may be present physiologically as synovial fluid, nutrients and wastes transfer across the face of chondrocytes. This has been simulated by *in vitro* experiments using the bioreactors, such as rotating-wall, perfusion and spinner flask bioreactors.[44]

One of the first studies of the impact of contact shear on cartilage tissue chondrogenesis studied bovine chondrocytes cultured in disks on polysulfone chambers.[45] The disks were exposed to shear strain amplitudes ranging from 1–3% and frequencies of 0.01–1.0 Hz for 24 hours in total, in order to determine the optimal conditions for proteoglycan and protein synthesis. Results indicated that there were no significant differences in proteoglycan or protein synthesis across these ranges, with a 25% and 50% increase respectively relative to static controls.45 A following study assessed the impact of long-term shear strain on bovine chondrocytes cultured on a porous calcium polyphosphate substrate.[46] In 4-week cell culturing experiments, the chondrocytes were exposed to a range of amplitudes and durations of shear strain, and the optimum condition of 2% shear strain amplitude at a frequency of 1 Hz for 400 cycles on alternate days was found. Under this optimum condition, after 1 week, collagen production had increased by 23% whilst proteoglycan production had increased by 20% relative to unstimulated controls. After 4 weeks, there was substantially more tissue grown (1.85 *vs.* 1.58 mg dry weight) and improved mechanical properties (load bearing capacity increased three-fold, stiffness increased 6-fold) relative to unstimulated controls.

Another study examining the influence of contact shear on human chondrocytes was conducted by Shahin and Doran.38 Healthy human foetal chondrocytes were isolated, then seeded onto polyglycolic acid-alginate scaffolds. These constructs were placed within the bioreactor and exposed to a combined compression and shear loading at a strain amplitude of 2.2%

(frequency = 0.05 Hz) for 10 minutes each day for 2.5 weeks. Results demonstrated a 2.1-fold increase in the construct's dry weight and a 2.0-fold increase in cell number relative to static control. There were also significant increases in GAG, total collagen, and type II collagen concentrations relative to the non-stimulated control.

Some other *in vitro* CTE studies also applied fluid shear stress on chondrocytes in the experiments. For example, Gooch et al.[47] determined the effect of fluid shear on ECM production of bovine calf chondrocytes. In this study, chondrocytes were seeded into polyglycolic acid disc-shaped scaffolds which were placed in spinner flasks and subjected to different angular velocities (0, 80, 120, and 160 r/min). It was found that scaffolds stimulated by fluid shear exhibited higher levels of collagen and generated and released more GAG relative to static scaffolds, with no significant variations between these intensities of angular velocities. Another study used human articular chondrocytes; it was found that fluid shear of 1.6 Pa applied using a cone viscometer altered chondrocyte morphology over a period of 48–72 hours.[48] The research observed that chondrocytes began to elongate and align in the direction of the fluid shear, and GAG synthesis increased 2-fold.[48] In another chondrocyte mechanobiological study, human articular chondrocytes were used, and exposed to fluid shear stress via a rotating-wall-vessel (rotating speed = 6 – 8 r/min).[49] Akmal et al.[49] found that GAG and hydroxyproline synthesis alongside type II collagen were significantly increased, compared to a static culturing condition.

Effect of hydrostatic forces on cartilaginous tissue growth

Hydrostatic forces can be defined as the forces exerted on the cell as a result of fluid pressure, either static or dynamic. One of the early studies in this field exposed primary bovine chondrocytes in a monolayer to 10 MPa of hydrostatic pressure for 4 hours.[6] In one group of cell culturing experiments, dynamic pressure with the frequency of 1 Hz was applied, while the other group was under static culturing condition. Results demonstrated that while static pressure had no effect on aggrecan or type II collagen mRNA levels, intermittent pressure increased these expressions by 31% and 36% respectively. Static pressure increased GAG synthesis by 32%, but intermittent pressure increased synthesis by 65%.[6] Additional studies of primary bovine chondrocytes suggested that intermittent pressures of 5–15 MPa promoted ECM production whereas continuous higher pressures of 20–50 MPa resulted in decreases.[50]

Studying human osteoarthritic chondrocytes cultured on type I and III collagen membranes, Scherer et al.51 found that biosynthetic activity was increased with dynamic hydrostatic pressure of 0.2 MPa following a loading regime of 30 minutes on and 2 minutes off. Furthermore, Ikenoue et al.[52] studied the impact of different regimes of intermittent hydrostatic pressure on normal human chondrocytes cultured in monolayer. In this study, cell culturing was carried out under hydrostatic pressures of 1, 5, and 10 MPa at 1 Hz for 4 hours per day. Aggrecan and type II collagen mRNA levels were analysed on days 1 and 4 and compared to unloaded cultures. Aggrecan signals increased by a non-significant amount, 30%, and 50% at 1, 5 and 10 MPa respectively on day 1, and increased by 40%, 80%, and 90% respectively on day 4. Type II collagen production was more sensitive to duration of exposure to dynamic hydrostatic pressure, and significant upregulation of mRNA signal was only found on day 4, with increases of 20%, 60%, and 70% for intermittent pressures of 1, 5, and 10 MPa respectively. This study provided clear evidence that separate markers of chondrogenesis may be influenced differently by hydrostatic pressure regime variations.[52]

In 2012, Correia et al.[53] used human nasal chondrocytes for studying the influence of hydrostatic pressure on chondrogenesis as evidenced by gene expression of aggrecan, type II collagen, and Sox9 as well as immunostaining of cartilage ECM. Chondrocytes were encapsulated in gellan gum hydrogels, cultured over a period of 3 weeks, and exposed to three stimulation regimes: (i) pulsatile hydrostatic pressure of 0.1–0.4 MPa at 0.1 Hz for 3 hours a day, 5 days a week; (ii) steady hydrostatic pressure at 0.4 MPa for 3 hours a day, 5 days a week; (iii) static culturing. It was found that the pulsatile regime induced an increase in the deposition of type II collagen and GAG relative to the steady hydrostatic pressure and static culturing conditions. Furthermore, the expression of type II collagen and *Sox9* genes were significantly greater than the steady and static controls.[53] In 2017, Nazempour et al.50 utilised a novel bioreactor system to simultaneously apply shear stress (0.02 Pa) and oscillating hydrostatic pressure (4 MPa at 0.5 Hz) to bovine articular chondrocytes in culture on agarose scaffolds for 21 days. They found that GAG and total collagen secretions were increased more significantly under a combination of shear stress and oscillating hydrostatic pressure together than that under either static condition or shear stress.[50]

In silico modelling for tissue engineering under dynamic condition

Bioreactors have been used for applying mechanical stimulation as their operation and physical environment can be accurately controlled, giving a comprehensive understanding of the forces acting upon the cells. *In silico* approaches have been used to calculate cell stimulation under dynamic conditions and/or determine the loading conditions of bioreactors. Such an approach has the potential to greatly reduce the environmental impact of the research by cutting down on unnecessary, flawed, or problematic experiments, allowing for a more informed approach.

For bioreactors that apply a deformation to the scaffold, compression or stretching, the finite element method can be used to calculate the local strains. It is assumed that the cells are subjected to the strain magnitude at the location of the scaffold that they are attached to.[54]

For bioreactors that use medium flow with induced WSS for cellular stimulation, the computational fluid dynamics (CFD) approach has been used to analyse the generated fluid velocity and WSS.[44, 55] In cell culturing experiments using 3D porous scaffolds, it was found that the scaffold porosity, pore size and pore shape have a distinct influence on the resultant WSS acting on cells within bioreactors.[9] To quantify the flow velocity and WSS on cells within scaffolds, and/or to determine the loading

conditions of bioreactors, in most circumstances, the CFD models were based on empty scaffolds for WSS calculation at the scaffold surfaces. This was under the assumption that the WSS at the scaffold surfaces was a good representation of the WSS sensed by the cells that attach to the scaffold surfaces. This assumption is well met in the initial situation in cell culturing experiments where the cell morphology is flat once they are attached to the scaffold surfaces. A major limitation of these *in silico* models is their failure to consider the cells and developing tissue within scaffolds. As mentioned above, this is reasonable at the start of the experiment when the cells initially attach to the scaffold surface. However, as the cells grow and proliferate they produce ECM, and this will lead to a change in the scaffolds porosity and the surface morphology. Consequently, the fluid flow and WSS can dramatically change.[56, 57]

To account for the change in scaffold porosity, some *in silico* models included a tissue growth model based on a modified level set method that considered the influence of scaffold structs curvature κ and cellular WSS τ (Equations 2 and 3). Guyot et al.[58, 59] simulated the dynamic process of neo-tissue growth V_G based on this model:

$$V_G = \begin{cases} -A \cdot \kappa \cdot f(\tau)\ \kappa > 0 \\ 0\ \kappa \leq 0 \end{cases} \quad (2)$$

$$f(\tau) = \begin{cases} 0.5 + \frac{0.5\tau}{a_1}\ 0 \leq \tau \leq a_1 \\ 1\ a_2 \leq \tau \leq a_1 \\ \frac{\tau - a_3}{a_2 - a_3}\ a_2 \leq \tau \leq a_3 \\ 0\ \tau \geq a_3 \end{cases} \quad (3)$$

where, a_1 and a_2 are the minimal and maximal shear stress enhancing neo-tissue formation (a_1 = 10 mPa, a_2 = 30 mPa) and a_3 the critical shear stress (50 mPa).[58]

Some other studies have defined that the tissue growth function V_G is governed by both the nutrient concentration C and the WSS as Equation 4:[60, 61]

$$V_G = \frac{k_M \cdot C}{k_M + C} \cdot f(\tau) \quad (4)$$

wherein, k_M is the maximum cell growth rate, k_S is the saturation coefficient of nutrient concentration (e.g., k_M = 5.8 × 10^{-6} g/cm^3/s, k_S = 2.3 × 10^{-3} g/cm^3 for CTE application), a_1 and a_2 are the minimal and maximal shear stresses for enhancing tissue formation (e.g., a_1 = 100 mPa, a_2 = 600 mPa for cartilaginous tissue formation) and a_3 the critical shear stress value (e.g., a_3 = 1000 mPa for cartilaginous tissue formation). In Equation 5, the WSS τ is calculated from the CFD model, and the nutrient concentration C is calculated from nutrient the diffusion-convection model, which moreover is coupled with the CFD model for the fluid velocity in convection term:

$$f(\tau) = \begin{cases} 0.6 + 4\tau\ 0 \leq \tau \leq a_1 \\ 1\ a_1 \leq \tau \leq a_2 \\ 2.5(1 - \tau)\ a_2 \leq \tau \leq a_3 \\ 0\ \tau \geq a_3 \end{cases} \quad (5)$$

Some tissue engineering experiments used mechanical compression to the scaffold in the bioreactor for applying mechanical stimulation (mechanical strain) on cells.[62] Previous *in silico* models have simulated the cell differentiation, proliferation, migration and apoptosis of cells within scaffolds under mechanical compression:[63, 64]

$$\frac{\partial n_i}{\partial t} = D_h \cdot \nabla^2 n_i + f(p) \cdot \nabla n_i + f^{PR}(S) \cdot n_i - f^D(S) \cdot n_i - f^A(S) \cdot n_i \quad (6)$$

where, S is the mechanical stimuli that depend on the octahedral shear strain and interstitial fluid velocity inside neo-tissue; ni was the cell densities of different cell phenotypes i, e.g., multipotent stem cells, and chondrocytes.; D_h is the cell migration rate; $f(p)$ is a function of fluid pressure (p); $f^{PR}(S)$, $f^D(S)$ and $f^A(S)$ are the mechanical stimuli (S)-related cell proliferation, differentiation and apoptosis rates, respectively. This *in silico* model for predicting cellular activities under mechanical compression was not developed explicitly for facial CTE. However, the model can be applied for CTE when the parameters, such as Dh, $f^{PR}(S)$, $f^D(S)$ and $f^A(S)$ are tuned to fit the context of facial CTE.

After further development and adaption of the *in silico* models for the application of CTE, these *in silico* models may help in optimising the experiment conditions for facial CTE, avoiding too high cost in terms of time and finance caused by excessive trial-and-error experiments.

Conclusion and Outlook

Using a combination of *in vitro* and *in silico* approaches can elucidate and optimise the micro-mechanical environment of chondrocytes for CTE. Although big progress has been made in the mechanobiology of chondrocytes for articular CTE, it is still a marginal area in facial CTE. Another limitation of this review is that the current *in silico* models are not developed explicitly for facial CTE. However, similar types of mechanical stimulation/environment are considered in the *in silico* models in this review. For future applications in facial CTE, the parameters in the models will need to be adapted. Therefore, the authors expect that the information from this review can inform and guide the mechanobiology study for facial CTE. The following suggestions are made for future facial cartilage CTE studies:

• Replicate the mechanical stimulation types in cell culturing experiments for facial cartilage CTE using different bioreactors for perfusion, mechanical compression, and hydrostatic pressure. Thus, finding the optimal level of mechanical stimulation for specific purposes such as accelerating chondrocytes proliferation in facial CTE;

• Tune the mechanical properties of biomaterials for guiding the cell behaviours for facial cartilage regeneration, e.g., stiffness of cross-linked hydrogel for 3D bioprinting and stiffness of scaffold struts;

• If using scaffold-based techniques combined with bioreactors for dynamic cell culturing, scaffold porous geometric features such as porosity, pore size and pore shape can be designed for tuning the mechanical environment applied to cells for facial CTE.

To investigate the above issues based on *in vitro* experiments, numerous trial-and-error experiments will be needed, leading to high costs in terms of time and finance. Moreover,

without computing, quantitative data, e.g., for mechanical stimulation, scaffold geometries and mechanical properties of biomaterial, are difficult to obtain from *in vitro* experiments. With the validated *in silico* models, it is expected to (i) replace some of trail-and-error *in vitro* experiments for finding the optimal conditions for CTE; (ii) provide the precise values of parameters that need to be assessed. Therefore, these studies are suggested to be conducted using *in silico* – assisted *in vitro* investigation for generating rigorous and comprehensive understanding, meanwhile saving the cost of excessive trial-and-error *in vitro* experiments.

Author contributions

RJ and TL equally contributed to this narrative review, and drafted this review. RJ, TL, YX and FZ participated in the plan and design of this review. YX, CW and FZ read and edited the paper. All authors approved the final version of the manuscript.

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Conflicts of interest statement

None.

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