Mechanical stimulation to stimulate formation of a physiological collagen architecture in tissue-engineered cartilage: a numerical study

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The load-bearing capacity of today’s tissue-engineered (TE) cartilage is insufficient. The arcade-like collagen network in native cartilage plays an important role in its load-bearing properties. Inducing the formation of such collagen architecture in engineered cartilage can, therefore, enhance mechanical properties of TE cartilage. Considering the well-defined relationship between tensile strains and collagen alignment in the literature, we assume that cues for inducing this orientation should come from mechanical loading. In this study, strain fields prescribed by loading conditions of unconfined compression, sliding indentation and a novel loading regime of compression–sliding indentation are numerically evaluated to assess the probability that these would trigger a physiological collagen architecture. Results suggest that sliding indentation is likely to stimulate the formation of an appropriate superficial zone with parallel fibres. Adding lateral compression may stimulate the formation of a deep zone with perpendicularly aligned fibres. These insights may be used to improve loading conditions for cartilage tissue engineering.

Keywords: cartilage; tissue engineering; agarose; collagen architecture; finite element modelling

1. Introduction

Osteoarthritis is a common pathology for which no satisfactory treatment exists. A promising treatment is to replace damaged cartilage with tissue-engineered (TE) cartilage (Risbud and Sittinger 2002; Kuo et al. 2006; Nesic et al. 2006; Noth et al. 2008). However, the insufficient load-bearing capacity of today’s TE cartilage is an important limiting factor in its clinical application. The mechanical properties of native articular cartilage are determined by two constituents in its ultrastructure: proteoglycans and collagen fibres. Proteoglycans attract water through osmotic pressure, which allows cartilage to withstand high compressive forces. Collagen resists tension and the specific depth-dependent organisation of collagen in cartilage (i.e. vertical fibres in the deep zone and horizontal fibres in the superficial zone) is optimised for distributing loads.

One of the main challenges for tissue engineering of mechanically stable cartilage is, therefore, to find the cues to create an engineered tissue with an ultrastructure similar to that of native tissue. A well-established cue for improving the mechanical properties of TE cartilage is mechanical stimulation (Davidson et al. 2002; Lima et al. 2007). To mimic the in vivo compressive loading condition, the commonly used loading condition is dynamic uniaxial confined or unconfined compression, which enhances matrix synthesis and produces matrix with better properties than those of unloaded tissue (Mauck et al. 2000, 2003; Kelly et al. 2006). The pressure presumably stimulates cells to synthesise proteoglycans, the function of which is to resist pressure, while tension presumably is a stimulus for cells to generate collagen fibres, the function of which is to resist tension. With these commonly used loading conditions, it is possible to tissue engineer cartilage with almost native proteoglycan content in 5 weeks of culture. However, collagen reaches only 15–35% of the native content after 5–12 weeks (Hu and Athanasiou 2006; Miot et al. 2006; Eyrich et al. 2007). Furthermore, an arcade-like collagen network is not reproduced in engineered cartilage. For these reasons, sufficient load-bearing properties are not reached (Miot et al. 2006; Yamaoka et al. 2006).

The functional significance of the arcade-like collagen structure is well emphasised in the literature (Korhonen et al. 2002; Owen and Wayne 2006; Wilson et al. 2007; Korhonen et al. 2008; Shirazi and Shirazi-Adl 2008; Shirazi et al. 2008). Vertical fibrils in the deep zone protect the solid matrix against large strains at the subchondral junction (Shirazi and Shirazi-Adl 2008). The superficial zone plays a crucial role in resisting elevated tensile stresses parallel to the articular surface (Owen and Wayne 2006). Furthermore, higher tensile modulus and lower compressive modulus at the articular surface promote interstitial fluid pressurisation, beneficial for cartilage lubrication (Krishnan et al. 2003; Kelly et al. 2006). Considering this emphasis on the arcade-like collagen architecture in load-bearing properties of native cartilage, it is logical to assume that without the reproduction of such
collagen architecture in engineered tissue, implanted constructs may have little chance of survival in vivo. However, this field of investigations is not fully mature (see review articles: Responte et al. 2007; Klein et al. 2009). Approaches such as using depth-dependent scaffold properties or cell sources have shown limited success (Kim et al. 2003; Malda et al. 2005; Ng et al. 2005, 2009; Klein et al. 2007; Moutos et al. 2007).

We propose that applying appropriate physical signals and mechanical conditions on cells during cartilage TE culture period can result in sufficient collagen synthesis and also in the production of an arcade-like collagen network. In native tissue, physiological joint loading is essential for the development and maintenance of normal articular cartilage. During development, both movement and mechanical loads play a critical role in differentiating embryonic mesenchymal stem cells into chondrocytes leading to the development of the articular surface (Arokoski et al. 2000; de Rooij et al. 2001; Nesic et al. 2006). Recently, a loading regime involving indentation with subsequent sliding of the indenter has been proposed assuming that this loading regime can provide a mechanical condition closer to that in native tissue, compared to common confined and unconfined compression loading conditions, and this can be a possible cue for stimulating cells to produce sufficient matrix (Bian et al. 2009; van Donkelaar et al. 2009). However, a critical appraisal of the mechanical conditions induced by this loading regime has never been published except for experimentally oriented papers (Kock et al. 2010) or conference abstracts (van Donkelaar et al. 2009; Khoshgoftar et al. 2010). To investigate the effect of different aspects (e.g. indentation depth or indenter and container shapes) and to examine different possibilities experimentally are labour intensive. Theoretical and computational modelling can be a powerful yet cost-effective alternative.

Theoretical models to predict the collagen network architecture in various tissues are based on the principle that if collagen’s function is to resist tension, then collagen aligns in the direction in which it can most efficiently perform this function, i.e. it will align with a direction that is determined by the magnitudes and directions of the positive principal strain (Driessen et al. 2003). This principle nicely explains collagen structures in uniaxially loaded tendons and in tissues that receive more complex loads [e.g. heart valves (Driessen et al. 2003), blood vessels (Driessen et al. 2004) and articular cartilage (Wilson, Huyghe et al. 2006)]. These concepts have been applied successfully to design loading protocols for tissue engineering of, for example, heart valves (Mol et al. 2006).

Our aim is to apply this approach to evaluate common and newly proposed loading protocols for engineering of cartilage, and to propose a protocol that would enhance collagen synthesis and to potentially stimulate the formation of an arcade-like structure. We analyse the strain fields under different loading conditions, seeking for a regime that produces relatively large positive strains, which are oriented such that the positive principal strains together constitute an arcade-like strain field. These loading conditions are unconfined compression as a common loading regime, sliding indentation as the newly proposed regime (Bian et al. 2009; van Donkelaar et al. 2009) and a combination of sliding indentation with lateral compression. We target agarose–chondrocyte constructs, commonly used for cartilage tissue engineering (Buschmann et al. 1992; Chowdhury et al. 2004; Ng et al. 2005; Kelly et al. 2006; Lima et al. 2007).

2. Theory and simulation

We hypothesised that if, indeed, positive principal strains define the collagen orientations, then the induced strain field by the TE loading protocol is indicative of the fibre structure that develops. To predict the induced strain fields generated by different loading regimes, we used the finite element method.

It has been shown that a hyperfoam or modified Ogden-Hill material model can describe the mechanical behaviour of highly compressible nonlinear elastomeric foams (Schrodt et al. 2005; Petre et al. 2006). Agarose hydrogel behaves as a compressible nonlinear poroelastic material (Buschmann et al. 1992). It has been shown that a hyperfoam model with strain-dependent permeability can accurately describe the agarose nonlinear poroelastic behaviour (Muralidharan 2006). The modified Ogden-Hill strain energy potential (hyperfoam) is available in ABAQUS 6.7 (SIMULIA, Providence, RI, USA).

The constitutive relation for a hyperfoam material was described as

\[
W = \sum_{i=1}^{N} \frac{2\mu_i}{\alpha_i} \left[ \lambda_1^{\alpha_i} + \lambda_2^{\alpha_i} + \lambda_3^{\alpha_i} - 3 + \frac{1}{\beta_i} (J^{-\alpha_i\beta_i} - 1) \right],
\]

(1)

where \( W \) is the strain energy density function and \( \mu_i \), \( \alpha_i \) and \( \beta_i \) are the unknown material constants. The deformation modes are characterised in terms of the volume ratio \( J \) and the principal stretches, \( \lambda_1, \lambda_2, \lambda_3 \). \( N \) is the order; for agarose \( N = 2 \) (Muralidharan 2006).

Strain-dependent permeability was implemented as (Wilson, Driessen et al. 2006)

\[
k = k_0 \left( \frac{1 - n_f}{1 - n_{f0}} \right)^M,
\]

(2)

where \( k_0 \) is the initial permeability, \( M \) is a positive constant, and \( n_f \) and \( n_{f0} \) are the current and initial fluid fractions, respectively. Material parameters of hyperfoam
model for 2\% (w/v) agarose are derived from the literature (Table 1). Hydraulic permeability parameters are $k_0 = 2.65 \times 10^{-12}$ (m$^2$/N s) and $M = 4.1$ (Muralidharan 2006).

### 2.1 Unconfined compression

An axisymmetric finite element mesh was used, consisting of 1500, eight-node linear axisymmetric pore pressure elements (CAX8P) (Figure 1(a)). The construct was considered to be 2.38 mm in radius and 2.3 mm in height (Kelly et al. 2006). The displacements of the nodes at the symmetry axis were confined in the radial direction. The nodes on the sample lateral edge were prescribed to zero pore pressure to simulate free fluid flow. Given that the friction coefficient between the construct and the impermeable plates falls in the range of 0.001–0.1 (Gong et al. 2000; Gong 2006), upper and lower limits of this range were considered to model the contact using a penalty approach. Two per cent strain was initially applied to the constructs in 1000 s followed by 10\% strain at a frequency of 1 Hz (Kelly et al. 2006).

### 2.2 Sliding indentation

During sliding indentation, an indenter compressed the sample and moved over the sample (Figure 1(b)) (Bian et al. 2009; van Donkelaar et al. 2009). A 3D finite element mesh consisting of 48,000 four-node pore pressure elements was used. The indenter ($\phi = 4.5$) was modelled as a rigid body. The sample was enclosed in a second rigid body, the container. The construct was considered to be 12 mm in the longitudinal direction, 3 mm in the vertical direction and 9 mm in out-of-plane direction.

<table>
<thead>
<tr>
<th>Material parameter ($N = 2$)</th>
<th>$\mu_i$ (MPa)</th>
<th>$\alpha_i$</th>
<th>$\beta_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$4.61 \times 10^{-2}$</td>
<td>7.512</td>
<td>$-0.1204$</td>
</tr>
<tr>
<td>2</td>
<td>$-3.68 \times 10^{-2}$</td>
<td>6.23</td>
<td>$-0.1940$</td>
</tr>
</tbody>
</table>

Table 1. Material parameters for agarose (Muralidharan 2006).

Figure 1. The applied loading geometry and boundary condition. (a) Unconfined compression, (b) sliding indentation; the construct is placed in a rigid container and the indenter moves over the construct in the X-direction. (c) Compression–sliding indentation; compressing post located at the side induces compression on the construct in the Z-direction and the sliding indenter moves over the construct in the X-direction.
For symmetry reason, only half of the construct was modelled (i.e. the out-of-plane length equals 4.5 mm). Contact between the container and the right and left sample edges as well as between the indenter and the sample was modelled using a penalty approach with friction coefficient between 0.001 and 0.1. Free fluid flow was allowed at the surfaces that were not in contact with the indenter or the container. During simulation, the indenter was first vertically displaced to 5 or 10% indentation depth and then was horizontally displaced 7 mm, back and forth in the longitudinal direction, at 2 mm/s.

### 2.3 Compression-sliding indentation

An intuitively more favourable condition to generate an arcade-like strain field was with a new configuration when a post located at lateral side induced compression on the construct when the indenter was sliding over the construct (Figure 1(c)). The construct was ‘semi-confined’ by the lateral compressing post. The rational was that deformation at the surface, where fibres parallel to the surface are desired, was unconfined, while in the deep zone, the material was deformed only in the vertical direction. The same mesh and boundary condition as for sliding indentation was used. Free fluid flow was allowed at the lateral edge of the construct which was not in contact with the compressing post. The construct was first compressed by the lateral post. Then, the indenter was vertically displaced to the desired indentation depth of 5% and then the sliding indenter was horizontally displaced 7 mm, back and forth in the longitudinal direction, at 2 mm/s.

### 3. Results

For unconfined compression (Figure 2), applying 10% dynamic compression generated tensile strains in in-plane and out-of-plane directions with a maximum of 4.6% when a friction coefficient of 0.001 was considered between the impermeable plates and the construct (Figure 2(a),(d)). Increasing the friction coefficient to 0.1 did not
significantly change the maximum strain values and only the strain profile was slightly changed (Figure 2(b),(e)). In-plane tensile principal strain was in the radial direction perpendicular to the loading direction (Figure 2(c)).

For sliding indentation, when the indenter compressed the construct, the region under the indenter experienced high tensile strains. At 5% indentation (Figure 3(a)), tensile strain reached 4.7% under the indenter in the longitudinal direction parallel to the surface. In the two lateral regions of the indenter, tensile strains with the direction of approximately 45° were generated. In the off-centre areas, vertical tensile strains with a lower value were generated. Increasing the indentation depth from 5 to 10% resulted in increased maximal principal strain from 4.7 to 7.0% (Figure 3(b)).

When the indenter was moved over the construct, a varying strain field was generated in the construct. To investigate the effect of friction between the indenter and the construct, the friction coefficient was increased from 0.001 to 0.1 at 10% indentation. This increase in friction did not significantly change the maximum principal strain magnitude and distribution, and only the area experiencing highest tensile principal strain was slightly larger (Figure 4). For 5% indentation, this effect was negligible (data not shown). Five per cent indentation with friction coefficient of 0.001 was considered for the rest of the simulations.

When the indenter was moved over the construct, the cyclic strain during the sliding indentation was not equal throughout the depth of the construct. The principal strains were large near the surface and strain magnitudes ameliorated in the deeper areas (Figure 5(a),(b)). Areas inside the construct located at different depths experienced a cyclic strain with different magnitudes and directions as shown for points located at the top (with height of 2.5 mm), middle top (with height of 2 mm), middle (with height of 1.5 mm) and middle bottom (with height of 1 mm) (Figure 5(b)). The maximum principal strain was generated with a distance to the surface, at the middle top area of the construct. A point located in this area (Figure 5(c)) experienced the highest tensile principal strain of around 6% when the indenter passed over this point during a sliding cycle. This highest tensile principal strain was approximately equal to the tensile strain in the

![Figure 3.](image-url)
longitudinal direction showing that the highest tensile strain occurred in the longitudinal direction. The compressive strain induced in the vertical direction was in the same range of magnitude.

For compression–sliding indentation, when the lateral compressing post applied 5% compression, vertical strains were generated in the deep zone of the construct reaching approximately 8% (Figure 6(a)). The superficial zone remained almost unstrained. When 5% compression was subsequently prescribed by the sliding indenter on top, the integrated maximal principal strain over time resulted in a deep zone with vertical tensile strain and a superficial zone with random and horizontal tensile strain. The maximal tensile principal strain occurred in the middle top depth reaching 8.3%. The superficial and deep zones are obvious (Figure 6(b)).

4. Discussion

Strain fields of different loading protocols have been investigated as the criteria to relate the mechanical loads, in particular the maximum principal strain directions, to the directional formation of collagen fibres in cartilage TE constructs. In addition to the fact that collagen structures in different tissues were correctly predicted based on this relationship (Driessen et al. 2003, 2004; Wilson, Huyghe et al. 2006), this important assumption is supported by in vivo experimental studies in native tissues (Nakatsuji and Johnson 1984) and in vitro TE studies (Kelly et al. 2006; Lee et al. 2008). In vivo experiments have demonstrated that uniaxial strains stimulate the alignment of collagen fibres in the tensile principal strain direction (Nakatsuji and Johnson 1984). In a cartilage TE study (Kelly et al. 2006), using polarised light microscopy, it has been shown that unconfined compression aligns collagen fibres perpendicular to the loading direction. This is in agreement with our prediction for unconfined compression where tensile strains perpendicular to the vertical compression were computed.

The strain field generated by applying unconfined compression loading regime may be useful to generate a superficial zone with collagen fibres parallel to the surface or higher modulus near the surface zones (Kelly et al. 2006). However, consistent with experimental observations, our prediction shows that a physiological collagen network with vertical fibres in the deep zone may not be produced by applying unconfined compression loading regimes, because vertical tensile strains are absent in that direction.

In the sliding indentation loading regime, varying the indentation depth can naturally change the strain field. It is apparent that when the depth of the indentation is increased, higher strains parallel to the surface will be induced deeper in the construct. This is an unwanted effect because vertical fibres are aimed for in these deeper zones. On the other hand, by increasing the indentation depth, larger vertical strains at off-centre areas, which are desirable, are induced. Yet, during one sliding cycle, at each point, the induced tensile strain parallel to the surface is much larger than the induced vertical tensile strain.

Figure 4. Distribution of the longitudinal logarithmic strain when the indenter moves over the construct at indentation depth of 10% with friction coefficient of 0.001 (top) and 0.1 (bottom).
Consequently, the sliding indentation is predicted to lead to produce an engineered tissue with undesired collagen network architecture in which collagen fibres are mostly directed parallel to the surface. However, due to the variation in the direction of the induced tensile strain, randomly oriented collagen fibres are also likely to be present in the produced collagen network.

In compression-sliding indentation, vertical strains are generated in the deep zone by means of the lateral compressing post. A small depth sliding indentation over
the construct induces a thin superficial zone. Therefore, this loading regime theoretically produces an arcade-like strain field with the vertical tensile strain in the deep zone and horizontal tensile strain in the superficial zone. One important benefit of this approach is that the thickness of the superficial and deep zones can be controlled by the adjustment of the indentation depth of the sliding indenter and compression applied by the lateral post. This is useful for creating TE cartilage with dedicated zone thicknesses. Besides, the effect of the transient development of the tissue can be controlled, adjusting the magnitude of the applied strains. For instance, the effect of elevating swelling strain as a result of the ongoing proteoglycan synthesis can be accounted for when adjusting the indentation depth and lateral compression during the culture period.

When sliding indentation is applied on the TE construct and the indenter moves back and forth over the construct surface, a highly varying strain field is generated in the construct. Cells embedded in the construct experience tensile strains with different magnitudes and in different directions. This highly inhomogeneous condition is different from the mechanical environment generated by the unconfined compression loading regime in which the cells are mostly deformed only in the direction perpendicular to the loading direction. We speculate that such varying strain field generated by the sliding indentation loading regime, in time, could enhance the metabolic and anabolic activity of chondrocytes. This, in part, may explain the enhanced collagen synthesis observed in the experiments (van Donkelaar et al. 2009; Kock et al. 2010). Such mechanotransduction processes of matrix remodelling are not yet understood. The effect of the variation in the strain direction in time is unknown. We do not know whether only the maximum strain direction is important or whether the orientation that is present for the longest duration is important. Thresholds for cells to respond to changing physical environment may exist (Lee et al. 2008) and it is unknown how these changing environments are integrated over time by the cells (Butler et al. 2000). As physiological loading in native cartilage is a complex combination of compression, tension, shear and fluid pressurisation, loading regimes which provide a condition closer to this in vivo condition may improve the development of a more biomimetic TE cartilage. For instance, in chondrocyte-seeded polyurethane constructs, the combination of axial compression and surface motion of a ceramic hip ball along the surface of the construct resulted in an increase in the mRNA and protein levels of surface zone protein, mRNA levels of hyaluronan synthase and in the release of hyaluronan (Lee et al. 2006). Although the sliding indentation concept is slightly different, we can expect similar results and it would be interesting to explore this experimentally.

This paper addressed the orientations of strain, assuming that these represent the ultimate orientation of collagen in the developing tissue. Most likely, the magnitudes of strains are important determinants for collagen synthesis as well. Indeed, it is not known between which strain boundaries collagen synthesis would be stimulated. Also, cell-independent effects on collagen metabolism are strain dependent (Huang and Yannas 1977; Nabeshima et al. 1996; Ruberti and Hallab 2005). Enzymatic degradation of collagen is minimal at 4% strain.

Figure 6. (a) The strain field produced by lateral compressing post applying 5% compression. (b) Maximal principal strain during a full cycle of sliding indentation with indentation depth of 5%; half of the construct is shown.
(Huang and Yannas 1977). Furthermore, strain of above a certain value may cause disruption. As shown in our study, the optimisation of the loading regime to induce a strain field with the desired direction and magnitude is possible. These regimes can now be used as input in the experimental research, which can then be used for the validation of our predictions. In this study, we evaluated the initial state of the TE construct when no matrix is produced in the engineered tissue. To fully optimise the loading protocol, transient development of tissue properties with ongoing matrix synthesis (Klisch et al. 2003) should be accounted for. One of our future aims is to use computer models to take such transient changes into account. Experimentally, monitoring matrix formation and comparing this with the applied strain fields will elucidate the cellular response to the strains. This can be used to further enhance the loading regime for cartilage engineering.

5. Conclusion

On the basis of strain fields that develop in a TE construct with different loading protocols, we suggest that the sliding indentation over a construct stimulates the formation of a superficial zone area. By inducing lateral compression, the formation of vertical fibres in the deeper zone can be stimulated. This effect can be enhanced when sliding is combined with compression. This theoretically results in the formation of a more physiological arcade-like collagen network. The present study shows how numerical simulations can be used to assist in evaluating and designing loading protocols for tissue engineering and provide useful insights for experimental studies to discriminate promising protocols from those with poor potential, which is a step forward towards successful tissue engineering of cartilage.

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