The tissue as self-assembly of notochord with sequential linear programming

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Abstract: Zebrafish serves as a model organism in morphogenesis studies in cell biology. One of the main difficulties of modeling a specific morphogenetic process come from the proper identification of its key features. To study the influence of geometric constraint in morphogenesis, here this article models the notochord as hard spheres assemble in an elliptical cylinder and optimize the packing density. This article implements linear approximation on the hard sphere constraints and find the optimal packing configuration by the sequential linear programming (SLP) method. During the process, this article modifies the SLP scheme into expanding the spheres in a fixed cylindrical confinement. This article finds the staircase and similar structures becomes predominant as increasing the ellipticity of the confinement. Our study deepens the understanding of the notochord morphogenesis, and also provides methodological guidance on related modeling studies involving geometric constraints.

1. Introduction
Packing problem has long been a considerably fascinating genre for mathematicians. It is an optimization problem that involves packing objects into a certain space. The goal of packing problem is to maximize the density of objects in infinite space or in a given container. In particular, Kepler (1611) made a well-known conjecture such that the maximum density fraction of sphere packing in 3-dimensional space is about 74.05%. About 100 years later, Newton suggested that the optimal number to surround a sphere with equal sizes of spheres is 12. The formalism of packing problems like these are simple, but the strict proof turned out to be far more complicated than expected. Newton’s assumption was proven in 1953, and not until 2014 was Kepler’s conjecture finally proven [1,2].

While packing in infinite space is directly connected to the lattice structures and modeling a huge amount of material, on the other hand, packing under confinement, plays an essential role in various disciplines from nano-manufacturing to cell biology. Nanomanufacturing itself is concerned with the packing and layering of nanoscale particles. For cell biology, for instance, scientists have related the packing problem with the selfassembly process of cells during the development of an organism from an embryo [3](sometimes named morphogenesis). Understanding the concept of packing may be relatively easy, but predicting the self-assembled structure with given confinement is much more challenging in both theoretical and practical contexts.

The morphogenesis of notochord is of particular interests in studies of morphogenesis mechanism [3–5]. The notochord is an elastic axial structure which extends through vertebrate organisms, and assembles the spine. It is composed of huge fluid-filled vacuolated cells that inflate
within an epithelial sheath that is encased in a thick extracellular matrix, and form into a stereotypical staircase pattern [3,6]. One of the major challenges in morphogenesis mechanism studies of a specific assembly process is to properly identify the most relevant physical quantities. As many physical and biological processes are spontaneously functioning in an organism, which one dominantly contributes to the morphogenesis is unclear. Fortunately, the computer modeling provides a technique to validate one proposed mechanism while excluding the influence of others [3,7]. For example, computer simulation has already been implemented to study the role of cell-to-cell interaction in notochord morphogenesis in early 1990s [8]; Varner et al studied the mechanical force in lung morphogenesis by mechanical modeling [7]. More recently, Norman et al considered the role of the geometry in notochord morphogenesis by modeling it as hard spheres packing within elliptical cylinders, then built both physical model and simulation model that optimized geometrical configuration by linear programming [3].

Figure 1: The microscopy image of zebrafish notochord morphogenesis. (upper) vacuolated cells in earlier stages and (down) the final arrangement. Scale bar = 25 µm. Picture from Ref. [3], Fig. S2.

Linear programming (LP) is a numerical method for optimization (such as maximizing profit or minimizing cost) that involves solving linear inequality systems. LP was developed independently by different scientists in early 20th centuries. One of the earliest records was that George B. Dantzig during 1946∼1947 implemented LP for planning problems in US Air Force [9]. Ever since then, LP has been employed in a variety of aspects in scientific researches. Formerly, LP only applied to the problems where constraints were all linear. When this prerequisite is not satisfied, however, one can first conduct linear approximation to the nonlinear constraints, then implement the so-called sequential linear programming (SLP) method for optimization. Torquato et al adapted SLP to find the optimal geometrical structure of particles under confinements in a microscopic system [10]. This method was implemented to find the dense packing of hard spheres packing within cylinders [11], which has shown significant improvement compared with earlier simulation works [12,13].

As mentioned before, the same method was recently implemented to model the notochord morphogenesis [3]. This work follows the same scheme as in Ref. [11] that shrinks the space in axial direction to achieve the close packing configuration, whilst vacuolated cells fulfill the cylindrical sheath by inflation. As the shape of sheath is a static confinement, the cell inflation process may also play a dynamical role in notochord morphogenesis. Here, we examine this hypothesis by modifying the SLP scheme into expanding the spheres in a fixed cylindrical confinement. The new scheme better describes the dynamical process of notochord morphogenesis. We describe the details of our methods in Section 2. In Section 3, we present and discuss our results, followed by a brief conclusion in Section 4.

2. Methods
The standard form of linear programming is to minimize \( f(x) = c^T x \) subject to \( A x \leq B \), where the column vector \( x \) contains the variables to optimize; the matrix \( A \) contains the prefactors of \( x \) and \( B \) is the constant terms in linear inequalities (constraints). Specifically, if we have \( n \) variables and \( m \) constraints, the LP problem defines as follows:
\[ \text{minimize } f(x) = (c_1 \ c_2 \ \ldots \ c_n)^T \cdot \begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{pmatrix} \]  

subject to

\[
\begin{bmatrix}
A_{11} & A_{12} & \cdots & A_{1n} \\
A_{21} & A_{22} & \cdots & A_{2n} \\
\vdots & \vdots & \ddots & \vdots \\
A_{m1} & A_{m2} & \cdots & A_{mn}
\end{bmatrix} \begin{pmatrix} x_1 \\ x_2 \\ \vdots \\ x_n \end{pmatrix} \preceq \begin{pmatrix} B_1 \\ B_2 \\ \vdots \\ B_n \end{pmatrix}
\]  

We now adapt the LP method for solving the dense packing of hard spheres confined in elliptical cylinder. Firstly, we denote an adapted cylinder coordinate system for sphere centers in elliptical cylinder as \((r_i, \theta_i, z_i)\), where

\[
\begin{align*}
    r_i & \in [0, 1] \\
    \theta_i & \in [0, 2\pi] \\
    z_i & \in [0, L]
\end{align*}
\]

which corresponds to

\[
\begin{align*}
x_i & \in ar_i \cos \theta_i \\
y_i & \in br_i \sin \theta_i \\
z_i & \in z_i
\end{align*}
\]

in Cartesian coordinate system. In other words, sphere centers can move within a cylinder whose cross section is an ellipse of semi-major axis \(a\) and semi-minor axis \(b\), where \(a \geq b\). Hence, we define the elliptical aspect ratio \(\lambda = a/b \geq 1\). When the equal sign is met, the ellipse reduces to a circle. Denote the diameter of hard spheres as \(d\), then the spheres are confined within circular cylinders of diameter \(D = 2a + d\).

The hard sphere constraint between sphere \(i\) and \(j\) is then written as

\[ (ar_i \cos \theta_i - ar_j \cos \theta_j)^2 + (br_i \sin \theta_i - br_j \sin \theta_j)^2 + |z_i - z_j|^2 \geq \left(\frac{d}{2}\right)^2 \]  

Note that \(|z_i - z_j|\) is calculated under the periodic condition

\[
|z_i - z_j| = |z_i - z_j - \frac{L \cdot \text{round}(z_i - z_j)}{L}|
\]

where round\((z)\) means that round \(z\) to the closest integer.

Because Eq. 5 is a nonlinear constraint, we shall conduct linear approximation to transform it into the standard LP problem and optimize it sequentially. Specifically, we assign each coordinate element with a small change such that \((r_i + \delta r_i, \theta_i + \delta \theta_i, z_i + \delta z_i)\), and insert it into Eq. 5 to obtain the prefactors for optimizing variables. For example, inserting

\[(r_i + \delta r_i) \Rightarrow r_i\]

\[a(r_i + \delta r_i) \cos \theta_i - ar_j \cos \theta_j)^2 + (br_i + \delta r_i) \sin \theta_i - br_j \sin \theta_j)^2 + |z_i - z_j|^2 \geq \left(\frac{d}{2}\right)^2 \]

and the prefactor for \(\delta r_i\) in this constraint is then

\[ A_{\delta r_i} = 2(a^2 \cos^2 \theta_i + b^2 \sin^2 \theta_i)r_i - 2(a^2 \cos \theta_i \cos \theta_j + b^2 \sin \theta_i \sin \theta_j)r_j \]

Other prefactors including \(A_{\delta r_j}, A_{\delta \theta_i}, A_{\delta \theta_j}, A_{\delta z_i}\) and \(A_{\delta z_j}\) are obtained in similar ways. Besides, the sphere diameter is expanded by \(\varepsilon_d\) such that \(d_{c+1} = d_c(1 + \varepsilon_d)\) in a single iteration. Consequently, the hard sphere constraint (Eq. (5)) is linearized as

\[ A_{\delta r_i} \delta r_i + A_{\delta r_j} \delta r_j + A_{\delta \theta_i} \delta \theta_i + A_{\delta \theta_j} \delta \theta_j + A_{\delta z_i} \delta z_i + A_{\delta z_j} \delta z_j + A_{\varepsilon_d} \varepsilon_d = B_{i,j} \]  

We shall also confine the change of coordinate elements in a single step within a small range such that
update the coordinate elements in every step until the convergence criteria are met. Hence, we have simplified our procedure by fixing the chiral double helices and triple helices appear successively and are separated by intermediate achiral structures [13]. The densest structure is a staircase (See Fig. 2). For example, when \( \hat{\rho} < 3/2 \), one sphere only touches with its two nearest neighbors in the densest packing at given \( \hat{\rho} \). In other words, one can predict the densest possible structure by finding the cross point of the state line given by Eq. (11) and the reference densest structure line in Fig. 2. During the expansion process, such that the packing fraction follows Eq. (11), as illustrated by the blue dashed lines in Fig. 2, until it reaches the axial number density of spheres and \( S_{cylinder} \) is the sectional area of the cylindrical container. In the case of circular cylinder, sphere centers are radially confined within a diameter of \( 2a \), so that \( S_{cylinder} = \pi(a + \frac{d}{2})^2 \), which also changes with \( d \). For convenience, we denote the ratio \( p = 2a/d \) then \( \eta \) depends solely on \( p \) during the expansion process, such that

\[
\eta = \frac{2}{3} \rho \frac{\pi d^3}{\pi (2a+d)^2} = \frac{4}{3} \frac{\rho a}{p(p+1)^2} \tag{13}
\]

Equation (11) suggests the packing fraction only depends on the scaled number density \( \hat{\rho} = \rho a \) and the scaled inverse sphere diameter \( p \). As one increases \( d \) (or equivalently, decreases \( p \)), the packing fraction follows Eq. (11), as illustrated by the blue dashed lines in Fig. 2, until it reaches the densest packing at given \( \hat{\rho} \). In other words, one can predict the densest possible structure by finding the crossing point of the state line given by Eq. (11) and the reference densest structure line in Fig. 2. For example, when \( \hat{\rho} < 3/2 \), one sphere only touches with its two nearest neighbors in the densest configuration. The densest structure is a staircase (See Fig. 4 and later discussions). As \( \hat{\rho} \) increases, the chiral double helices and triple helices appear successively and are separated by intermediate achiral structures [13]. When \( a \neq b \), the cross-section of the cylinder is an ellipse parallel curve [14]:

\[
\begin{align*}
    x &= \cos \theta \left( \frac{bd}{2a^2 \sin^2 \theta + b^2 \cos^2 \theta} \right) + a \\
    y &= \sin \theta \left( \frac{ad}{2a^2 \sin^2 \theta + b^2 \cos^2 \theta} \right) + b
\end{align*}
\tag{14}
\]
Figure 2: The maximal packing fraction ($\eta$) under different $p$ illustrated by the black solid line. Data obtained and units converted from Refs. [11,12]. The blue dashed lines show the packing fraction increases during increasing $d$ under a fixed $\hat{\rho} = 0.25, 0.5, 1, 1.5, 2, 2.5$ and 3, from bottom to above.

and the enclosed area is

$$S_{cylinder} = \left( aE \left( 1 - \frac{b^2}{a^2} \right) + bE \left( 1 - \frac{a^2}{b^2} \right) \right) + \pi (ab + \frac{d^2}{4})$$

(15)

where $E(m) = \int_0^{\pi/2} \sqrt{1 - msin^2\theta} d\theta$ is complete elliptic integral of the second kind. Then the packing fraction is

$$\eta = \frac{4}{3p} \cdot \frac{\pi \hat{\rho}}{\pi \hat{p}^2 + 2p \left( E \left( 1 - \frac{1}{\lambda^2} \right) + \frac{E \left( 1 - \lambda^2 \right)}{\lambda} \right) + \pi}$$

(16)

Eq. (14) suggests the packing fraction depends on $\hat{\rho}, \lambda$ and $p$. Clearly, Eq. (14) reduces to Eq. (11) at $\lambda = 1$. As the ellipticity of the boundary develops, the system approaches to the optimal configurations with different sphere diameters and of course, different packing fractions. We shall analyze the influence of boundary ellipticity, measured in aspect ratio ($\lambda$), in the following discussions.

Figure 3 illustrates that the staircase structure develops along with increasing the aspect ratio. For $\hat{\rho} = 1$, the densest packing configuration for the circular cylindrical confinement ($\lambda = 1$) is known as a double helix. As $\lambda$ increases, defects first appear in the helices, accompanied by the structure’s reorganization until a regular crossing-staircase structure is formed when $\lambda \geq 1.15$. This crossing-staircase can be viewed as two staircases located on two parallel planes, with each level consisting of two spheres and the levels are stacked alternately along the axial direction, resulting in a periodic structure of 4 spheres. These results are fully consistent with the previous study in Ref. [3].

For $\hat{\rho} = 1.5$, in contrast, the densest packing configuration for $\lambda = 1$ is a triple helix. The helicity disappears quickly as we increase $\lambda$—the structure for $\lambda = 1.05$ is already
Figure 3: Final configurations obtained from optimization for systems of (a) $\hat{\rho} = 1$ and (b) $\hat{\rho} = 1.5$ and under different elliptical aspect ratio. achiral and regular. It can be viewed as two staircases vertically crossing, where every other layer in each staircase orients slightly differently, thereby forming a periodic structure of 8 spheres. As we further increase $\lambda$, because the space is more constrained, the orientation difference of neighboring sphere pairs in both staircases vanishes, and the period reduces to 4 spheres as a result. This structure is essentially the same with the crossing-staircase in $\hat{\rho} = 1$ we have just described if we pair the spheres differently and get two parallel staircases (instead of vertical). The triple helices and the corresponding crossing-staircase structures in large $\lambda$ are also reported by Ref. [3]. Moreover, we first report the intermediate periodic structure of 8 spheres, which is part of a different mechanism for the development of crossing-staircase from triple-helices compared to that from double-helices.

![Figure 3: Final configurations obtained from optimization for systems of (a) $\hat{\rho} = 1$ and (b) $\hat{\rho} = 1.5$ and under different elliptical aspect ratio.](image)

Figure 4: Diagram of optimal configurations under different sphere density and elliptical aspect ratio $\lambda = 1$ and 1.3.

As $\hat{\rho}$ further increases, more complex dense packing structures appear. We summarize the typical sphere packing structures obtained in circular ($\lambda = 1$) and elliptical ($\lambda = 1.3$) cylinders in Fig. 4. For $\hat{\rho} = 2$ and 2.5, the optimizations give different structures in $\lambda = 1$, but they all reduce to the vertical crossing-staircase in $\lambda = 1.3$. However, for $\hat{\rho} = 3$, a quadruple-helix is formed in circular cylinders, and it still persists as far as $\lambda = 1.3$.

![Figure 4: Diagram of optimal configurations under different sphere density and elliptical aspect ratio $\lambda = 1$ and 1.3.](image)

Figure 5: The change of (a) packing fraction and (b) inverse scaled sphere diameter as increasing the elliptical aspect ratio. The legends denote the sphere density $\hat{\rho}$.

![Figure 5: The change of (a) packing fraction and (b) inverse scaled sphere diameter as increasing the elliptical aspect ratio. The legends denote the sphere density $\hat{\rho}$.](image)
Although defects are emerging. In summary, our results confirm that the cell packing by inflation in elliptical cylindrical confinement prefers the staircase structures and when the number densities are relatively small.

As a final mark, we quantitatively studied the change of packing fraction and the sphere diameter with elliptic aspect ratio. The results are shown in Fig. 5. In general, the packing fraction increases with $\lambda$, and the sphere diameter at the end of optimization decreases with $\lambda$ increasing. Note that the final sphere diameters are trivially constant for systems with $\bar{\rho} \leq 3/2$, as the densest structures are always staircase in the plane of major axis, thus independent with $\lambda$.

4. Conclusion
In this study, we have chosen to use expansion algorithm to determine how the change in packing density of cells and aspect ratio of the outer sheath leads to different packing results. We find that the staircase and similar structures become predominant as increasing the ellipticity of the confinement. Our results are consistent with previous knowledge and extend our understanding of the impact of geometric constraint in notochord morphogenesis. Moreover, our method is able to apply to a broader context, which is accessible not only to morphogenesis in cell biology, but also to general problems in reality related to the assembly controlled by geometric constraint.

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