Three-Dimensional Formation of Magnetic Micro-Gel Beads for Tissue Engineering

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Abstract

We have commenced basic research on the three-dimensional pattern formation of micro-gel beads for applications in biological tissue engineering. In this new technique, micro-gel beads are pre-magnetized by doping them with magnetic nanoparticles. Living cells will be included in beads for actual use. If a nonuniform magnetic field is applied to a solution containing these magnetized beads, the beads will align, contact, and form a 3D structure. The structure is controlled by the seed pattern of the magnetic particles plugged in a substrate and the profile of the magnetic field distribution. We have constructed tubes, which imitate blood vessels, for demonstration using gel beads whose diameters are of the order of several tens of micrometers. The diameter of the demonstrated tube was less than 0.5 mm and its length was 6.6 mm, although living cells were not included in the beads. Numerical calculations by using the discrete element method were conducted to confirm the formation of the tube and to predict the effect of centrifugal force, which will be applied to fill other tissues in the space between magnetically patterned beads. Although this unique technology is in the nascent stage, it can potentially be used to form three-dimensional, nonuniform, and heterogeneous artificial organs for tissue engineering.

Introduction

Tissue engineering has attracted considerable attention as a final therapeutic method for irreversible organ failure, for which no other treatment has been successful.[1] Scaffold-guided tissue regeneration is one of the key approaches to tissue engineering. This method involves seeding highly porous biodegradable scaffolds with donor cells and/or growth factors, followed by culturing and implanting the scaffolds to induce and direct the growth of new tissue. The goal is to first get the cells to attach to the scaffold and allow them to replicate, differentiate, and organize into normal healthy tissue as the scaffold degrades. Although this method has been used to create some uniform and homogeneous tissues including skin, cartilage, and bone, another new technology should be developed to create thick and large 3D tissues composed of different types of cells with a unique microstructure and with sufficient vasculature.[2-4]

In order to meet these requirements, we have started basic research on the 3D pattern formation of micro-gel beads. Living cells will be included in beads for actual use. In this new technique, micro-gel beads are pre-magnetized by doping them with magnetic nanoparticles; these magnetized beads are then aligned and contacted to form a 3D structure in a controlled magnetic field.[5] Although this unique technology is still in the nascent stage, it has the potential to form 3D, nonuniform, and heterogeneous artificial organs for tissue engineering.

Chain Formation in Magnetic Field

Magnetic particles form chains in a nonuniform magnetic field; this property of magnetic particles is utilized to form 3D cell structures.[6-8] Micro-gel beads were pre-magnetized by doping them with magnetic nanoparticles, as shown in Fig. 1. Thus far, living cells have not been included in beads, ever though this process is essential for the practical applications of these beads. Alginate micro-gel beads were formed by using the inkjet technology described in literature.[2] Two types of beads were prepared for experiments−A and B. Both types of beads had the same diameter and chemical components. Beads A contained small amounts of magnetic particles−2.5 mg/ml (nanomag-D silica, 250 nm, Micromod)−and their effective magnetic permeability was estimated to be only 1.003. On the other hand, beads B contained 125 mg/ml of magnetic nanoparticles, which is 50 times the amount of nanoparticles in beads A, and their effective magnetic permeability was estimated to be 1.15.

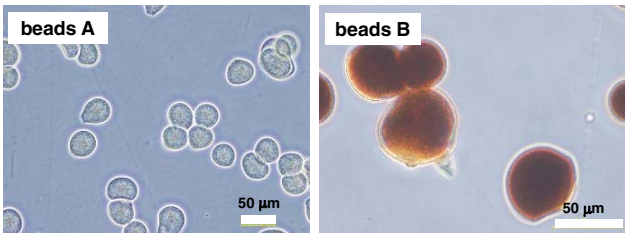

***Figure 1**. Photographs of micro-gel beads A and B magnetized by doping with magnetic nanoparticles.*

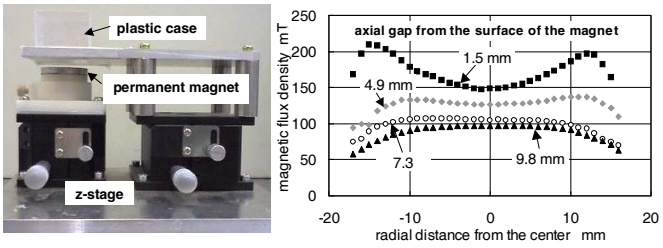

***Figure 2**. Setup to form magnetic gel bead chains (left) and distribution of magnetic flux density (right).*

As shown in Fig. 2, a plastic case filled with a solution containing beads A was mounted on a permanent magnet (Nd-Fe-B, 33.8 mm in diameter, 4.8 mm thickness, Neomax) that was used as a source of magnetic fields. The axial distance between the plastic case and the magnet was controlled manually by means of a z-stage in order to adjust the magnetic flux density in the case. The maximum magnetic flux density at the center of the case was approximately 150 mT, and it decreased as the distance from the permanent magnet increased.

Although the beads gathered near the edge of the permanent magnet, where the magnetic flux was concentrated, they did not form chains as shown in Fig. 3. It was assumed that this absence of chain formation was attributed to the low magnetic field or the extremely low effective permeability of the beads. A numerical calculation was made by using the distinct element method (DEM) in order to clarify this phenomenon. DEM is a numerical method for calculating the motion of a large number of particles based on Newton's laws of motion.[7-9] Figure 4 shows the chain formation of the magnetic gel beads obtained by varying the magnetic flux density and the magnetic permeability of the beads. Initially, 30 beads were placed at random positions in an area spacing 0.9 mm in width and 1.8 mm in height. Although the number of beads and the calculation domain were small, it was possible to estimate the outline of the chain formation. The motion of each particle was calculated every 10^{-7} s after the application of gravitational and magnetic fields; the profiles of the beads in the quasi-steady-state are shown in Fig. 4. Figure 4 (a) shows the effect of the magnetic flux density at the bottom of the chain, while Fig. 4 (b) shows the effect of the magnetic permeability of the beads. It can be clearly observed that chains are not formed even in an ultrahigh magnetic field of up to 10 T. On the other hand, from Fig. 4 (b), it was confirmed that the latter hypothesis–that the chain formation is highly dependent on the effective permeability of the beads–is relevant and that for chain formation, the effective magnetic permeability of the beads must be larger than 1.1. This hypothesis was confirmed experimentally as shown in Fig. 5, i.e., beads B, whose permeability is 1.15, formed chains even in a low magnetic field. Because the length of each chain varied widely, these lengths were fitted to the second-order regression curves, and the lengths of the chain at the center of the magnet were plotted as shown in Fig. 6 (marked as 'unseeded'). It was observed that the chain lengths increased with an increase in the magnetic field if the magnetic flux density was less than 0.02 T. As the field was increased further, the chain length was maximum at up to 0.04 T, following which the length decreased. This result quantitatively coincided with the preceding experimental and numerical results for solid spherical magnetic

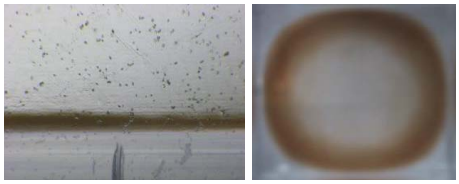

***Figure 3**. Gel beads A in a magnetic field 150 mT observed from the lateral side of the plastic case (left) and from above the plastic case (right). The dark circle in the photograph on the right corresponds to the circumference of the permanent magnet.*

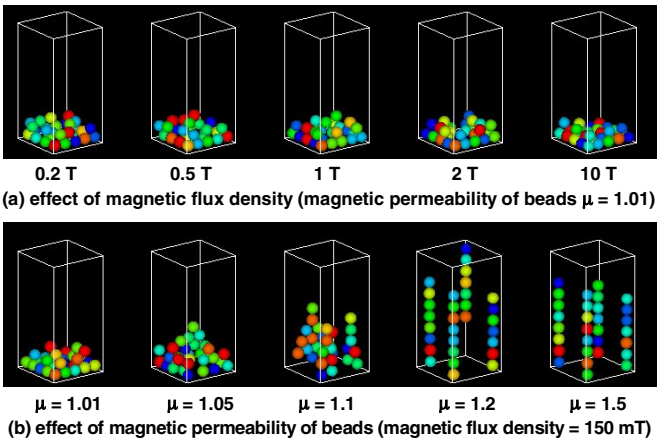

***Figure 4**. Calculated chain formations of magnetic gel beads.*

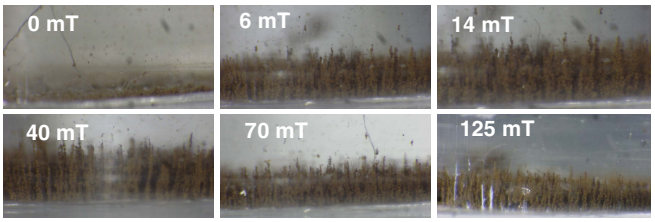

***Figure 5**. Chain formation of gel beads B in a magnetic field, observed from the lateral side of the plastic case.*

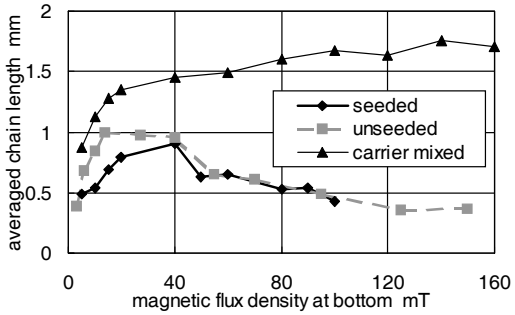

***Figure 6**. Relationship between the measured chain length and the magnetic flux density (gel beads B).*

beads.[8] In conclusion, it was deduced that the gel beads must contain sufficient magnetic nanoparticles to have high magnetic permeabilities of more than 1.1; however, a high magnetic field is not necessary for magnetic-field-dependent pattern formation.

3D Pattern Formation

Three-dimensional patterns of gel beads were formed in a premodulated magnetic field created by magnetic particles. This method is based on a preliminary investigation in which it was observed and calculated that chains of magnetic particles are formed on pre-seeded magnetic particles, as shown in Fig. 7. If small holes are drilled on a plastic substrate to form a matrix pattern and if magnetic particles are plugged in the holes, the chains can grow upward from the holes. The experimental result was confirmed by

a numerical calculation based on 3D distinct element method with a periodic boundary condition.[8] In the experiment, spherical soft magnetic particles (Toda Kogyo Corp., originally manufactured for use as carrier particles in a magnetic two-component development system for electrophotography) made of magnetite-doped phenol resin by the polymerization method were used; the average diameter of the particles was 88 μm, their volume density was 3,620 kg/m^3, and their relative magnetic permeability as obtained from the magnetization curve for the aggregation of particles was 4.7.[7, 8] Although solid particles were used for the preliminary investigation, it is expected that magnetized gel beads will also form the intended patterns with a similar method. Figure 8 shows the result of the experiment. The experimental procedure was as follows: (1) Small holes, 150 μm in diameter, were drilled on a 100-μm thick plastic sheet by a CO_2 laser beam to form the intended pattern. Two circular patterns, one 1.0 mm in diameter and the other 0.5 mm in diameter, were machined simply for demonstration. (2) Magnetic particles were plugged in the holes and covered by an adhesive cellophane tape. The particles used in this experiment were the same as those used in the preliminary experiment. (3) The sheet with plugged particles was placed at the bottom of the plastic case: (4) The solution with gel beads B was filled in the plastic case. (5) Finally, the case was placed on the permanent magnet. It can be clearly observed in Fig. 8 that the gel beads gathered on the pre-seeded magnetic particles and formed rings. The length of the pattern formed by varying the magnetic flux density was added to the length of the chain formed by gel beads on unseeded magnetic particles, and the results of the addition are shown in Fig. 6. Both the lengths were almost the same, and the maximum length of the pattern was 1 mm, which is too short for practical use.

An additional magnetic field was introduced to obtain an extended tube pattern. The setup is shown in Fig. 9. The plastic case filled with the solution containing gel beads B was mounted on the permanent magnet. The plastic sheet in which the patterned solid magnetic particles were plugged in was placed at the bottom of the plastic case. The procedure followed for forming 3D patterns in

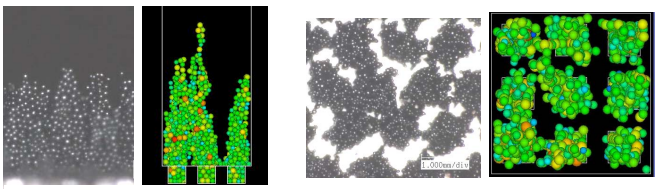

***Figure 7**. Formation of chains on pre-seeded magnetic particles (0.64 kg/m^2 surface loading, 18 mT).*

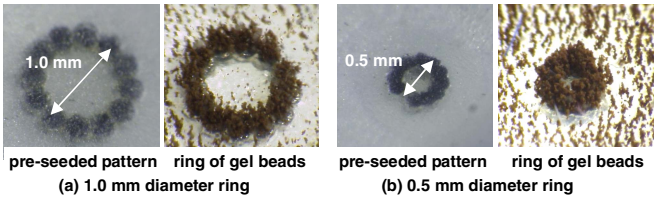

***Figure 8**. Formation of ring patterns of gel beads B on pre-seeded magnetic particles (40 mT).*

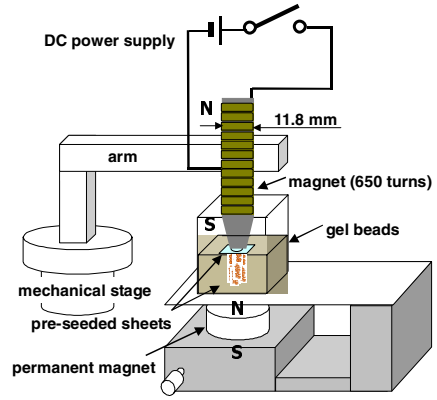

***Figure 9**. Setup to form long patterned magnetic gel bead chains.*

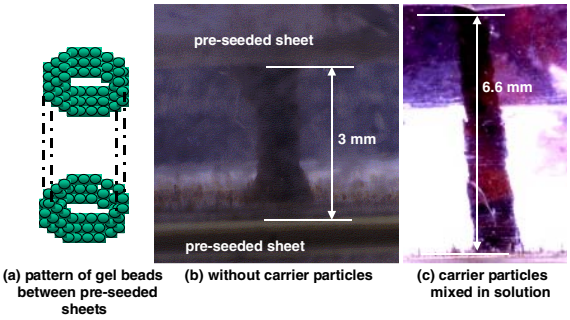

***Figure 10**. Prolonged bead pattern between pre-seeded sheets (gel beads B).*

this experiment was the same as that in the preceding experiment. An additional sheet in which the patterned solid magnetic particles were plugged in was placed 1.5 mm above the lower sheet. The upper and lower sheets were the same. The upper sheet was attached to the lower end of the electromagnet, which consisted of an ion core rod and a solenoid. DC voltage was applied to the coil to generate a magentomotive force of 800 AT. The magnetic flux density at the lower end of the pole was 123 mT. The electromagnet attached to the upper sheet was slowly turned upward manually by the mechanical stage connected to the electromagnet through an arm. Then, the length of the chain of gel beads increased up to 3 mm, as shown in Fig. 10 (b).

The calculated results shown in Fig. 4 (b) suggest that long chains will be formed if the magnetic permeability of the beads is larger than that of beads B. Carrier particles of permeability 4.7 were mixed in the solution instead of increasing the amount of magnetic nanoparticles in gel beads, because the amount of magnetic nanoparticles that can be doped in the gel beads is limited. The magnetic interaction force is expected to increase on mixing carrier particles in the solution. Figure 11 shows the averaged chain length with respect to the amount of mixed carrier particles and the averaged chain length in the case that 0.001 g of carrier particles were mixed in the solution is added in Fig. 6 (marked as 'carrier mixed'). The experimental procedure was the same to that of seeded with nanoparticles and unseeded. It was thus confirmed that long chains can be formed by mixing magnetic particles in the solution.

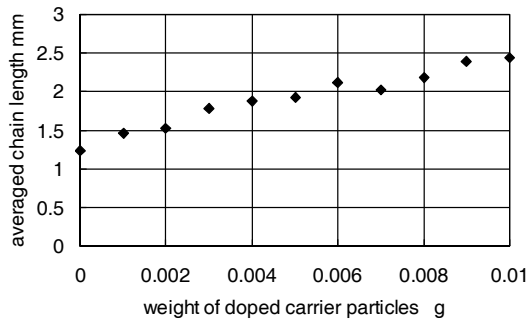

***Figure 11**. Relationship between measured chain length and weight of doped carrier particles (gel beads B, 40 mT).*

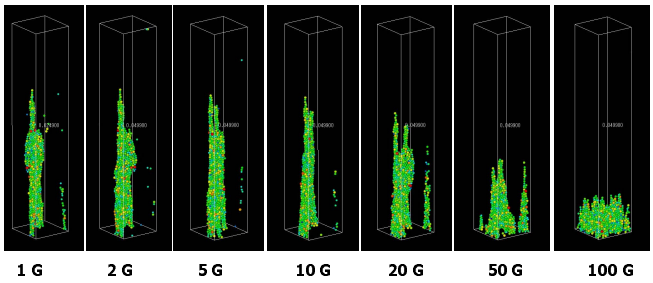

***Figure 12**. Effect of centrifugal force on magnetically formed chain of gel beads.*

Figure 10 (c) shows the demonstrated gel bead pattern in the sandwiched pre-seeded magnetic field with 0.001 g of carrier particles in the solution. A considerably long tube that simulates a blood vessel was formed.

After the intended 3D pattern of gel beads is formed, it is necessary to apply a high centrifugal force to fill other tissues in the space between the patterned chains and to form heterogeneous tissues. A numerical calculation was made to investigate the effect of the centrifugal force. Three-dimensional distinct element method with a periodic boundary condition was used for the calculation.[8] Figure 12 shows the calculated results. The weight of the gel beads was increased to the designated G value to simulate the high centrifugal field. It is predicted that the effect of the centrifugal force is negligibly small up to 10 G, but the chains are suppressed under an ultrahigh centrifugal field of more than 20 G. The centrifugal force must be limited to less than 20 G to maintain the magnetically formed pattern.

Concluding Remarks

A basic research on the 3D pattern formation of micro-gel beads was conducted for its potential applications to biological tissue engineering.

(1) Micro-gel beads were pre-magnetized by doping them with magnetic nanoparticles to utilize the magnetic force. The beads must contain sufficient magnetic nanoparticles to have a high magnetic permeability of more than 1.1, but a high magnetic field is not necessary for forming patterns dependent on the magnetic field.

(2) The pattern was controlled by the seed pattern of the magnetic particles plugged in a substrate and the profile of the magnetic field distribution.

(3) It was possible to prolong the gel bead pattern between pre-seeded magnetic fields in a solution containing magnetic particles.

(4) The centrifugal force required to fill tissues in the magnetically formed pattern must be limited to less than 20 G.

Although this unique technology is in the nascent stage, it can potentially be used to form three-dimensional, nonuniform, and heterogeneous artificial organs for tissue engineering.

The author wishes to express his sincere gratitude to Mr. Hiroki Inoue and Mr. Shyo Ohkado (Waseda University) for their help with carrying out the experiments, Prof. Makoto Nakamura (Tokyo Medical and Dental University) for his beneficial suggestions, and Dr. Shintaroh Iwanaga (Kanagawa Academy of Science and Technology) for supplying the gel beads used in the experiments. This work is supported by a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science and from the Nippon Sheet Glass Foundation for Materials Science and Engineering.

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