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| Original Article |

Fabrication of 3D Alginate Scaffold with Interconnected Pores using Wire-Network Molding Technique

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Abstract : In this study, we fabricated 3D porous scaffold by 'Wire-Network Molding' technique with alginate gel which has been used for cartilage regeneration because of the chemical similarity. Firstly, prepared ETPCS-S wires with size of rectangular cross section 600 μm by 600 μm, 400 μm by 400 μm, respectively, and the wires are inserted in designed mold. Secondly, sterilized 2 wt% alginate gel within hMSC (human Mesenchymal stem cell) was injected into the assembled mold. The concentration of hMSC in the used alginate gel is about 5000 cells per scaffold. For the gelation of alginate gel, the mold was soaked in 5 wt% $CaCl_2$ solution for 5 min. Subsequently, wires are separated from the mold and the mold is removed from alginate gel. Consequently, the remained alginate scaffold has interconnected pores with a configuration of wire-network. Additionally, to analyze the cell-culturing characteristics, 1-day, 3-day, and 7-day cultured scaffolds which encapsulate hMSC are assessed using MTS assay. Consequently, the optical density of 400 μm-WNM scaffolds and 600 μm-WNM scaffolds are clearly more increased than control scaffolds without pores.

Key words: *tissue engineering, scaffold, hydrogel, alginate, WNM (Wire-Network Molding)*

1. Introduction

For the disappeared or damaged tissue in human body because of accident or disease, tissue engineering could suggest a certain possibility to substitute the damaged/disappeared organ/tissue with an artificial organ/tissue without immunorejection.[1] For this reason, recently, many feasibility researches for the several tissue regeneration using scaffolds have been studied actively. Among tissues in human body, the cartilage is one of difficult tissues to be regenerated because it has no blood vessels and nerves.[2] For cartilage regeneration, several hydrogels have been used for the scaffold materials because of the chemical similarity to the extracellular matrix. Until now, the fabrication methods using hydrogel are proposed such as freeze drying,[4-8] fiber bonding,[9] 3D-plotter,[10] gas forming,[11] and electrospinning.[1,12] In the case of hydrogel scaffolds, the manufacturing of 3D scaffold with guaranteed interconnectivity is not reported except 3D-plotter technique.[10] However, 3D-plotter technique has some demerits which are the necessity of expensive equipment (Prices of commercialized 3D-plotter system are known as 100,000~150,000 dollars.) and the requirement of long manufacturing time. Moreover, according to the previous study,[10] the manufacturing of hydrogel 3D scaffold using 3D-plotter undergoes different gelation time with respect to the height of scaffold, because the hydrogel plotting is executed in the $CaCl_2$ solution. Therefore, the bottom part of scaffold is more soaked than the top part of scaffold. It could affect a certain damage to cells in the hydrogel or cause different mechanical and chemical characteristics between top and bottom of scaffold.

In this study, we proposed a novel method to fabricate 3D hydrogel scaffold encapsulated hMSC using 'Wire-Network Molding' technique. Wire-Network Molding technique was proposed by our team[13] and has several merits: guaranteed interconnectivity, effective in mass production, and low-cost.

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Additionally, because of the characteristics of the fabrication method, the gelation time is almost equal at top and bottom part of scaffold. In this study, 2 wt% alginate gel which has been used for cartilage regeneration commonly was used. The scaffolds are fabricated with 400 μm or 600 μm strand thickness using rectangular wires with 400 μm×400 μm or 600 μm×600 μm cross section, respectively. To analyze the cell-culture characteristics of fabricated scaffold, we assessed cell growth in 1, 3, and 7 days using MTS assay compared with control scaffold which has no interconnected pore.

2. Materials and Methods

2.1 Materials

In this work, frame used for outer wall such as Fig 1A was manufactured by Rapid Prototyping machine (Invision HR®). The used material is acrylic plastic (VisiJet HR-M100®). The outer frame could be manufactured according to the outer geometry which researchers need to fabricate. In this study, because we need a scaffold for the feasibility of our approach, the frame was manufactured with simple hexahedral shape. The metal wires depicted as orange-colored columns in Fig 1B, C, and D were used as sidewall components. The used metal wires (ETPCS-S) which has surface coated by 100% Tin on steel wire are supported by Hyunsung Electronics. The used metal wires have rectangular cross section with 400 μm×400 μm, 600 μm×600 μm, and 1000 μm×1000 μm such as Fig 1. Moreover, same wires depicted as green-colored columns in Fig 2 were used as networking frame such as Fig 2. For the scaffold material, alginic acid (viscosity= 20,000-40,000, Sigma-Aldrich) is used. Additionally, for the gelation of alginic acid, 5 wt% $CaCl_2$ solution which is made by Calcium chloride dehydrate powder (Sigma-Aldrich) is used.

2.2 Fabrication of Scaffold

2.2.1 Manufacturing a Mold for Hydrogel Scaffold

Fabrication process of well-interconnected 3D hydrogel scaffold using 'Wire-Network Molding' is as in the following. First, to make outer wall, base frame depicted as blue-colored structure in Fig 1A was manufactured by Rapid Prototyping machine (Invision HR®). After that, metal wires with rectangular cross section which are depicted as orange-colored columns in Fig 1 are used for the sidewall components as in Fig 1B, C, and D. The used metal wires have rectangular cross section with 400 μm×400 μm, 600 μm×600 μm, and 1000 μm×1000 μm, respectively. As shown in Fig 1B, C, and D, these wires were

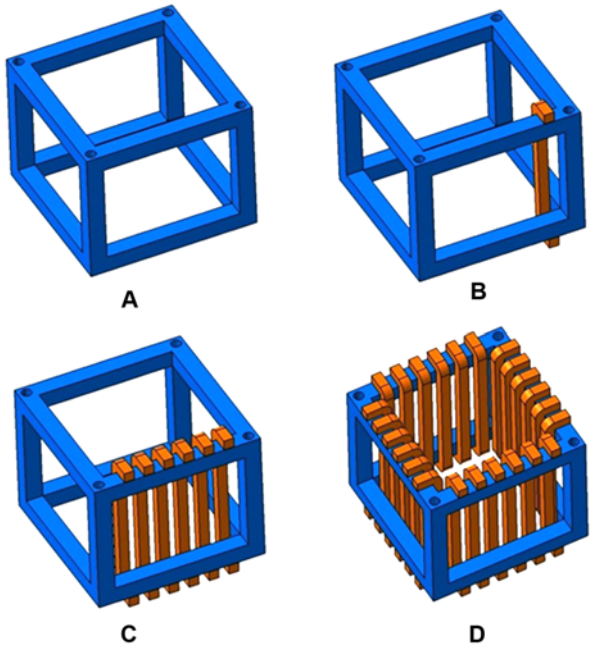

Figure 1. Assembling process of "outer wall" for wire-network molding technique: (A) "frame" manufactured by 3D printer, (B) and (C) sequential process of assembling "sidewall component" into "frame," (D) fabricated "outer wall" (blue-colored structure: frame, orange-colored structure: sidewall component which is metal wire having rectangular cross-section).

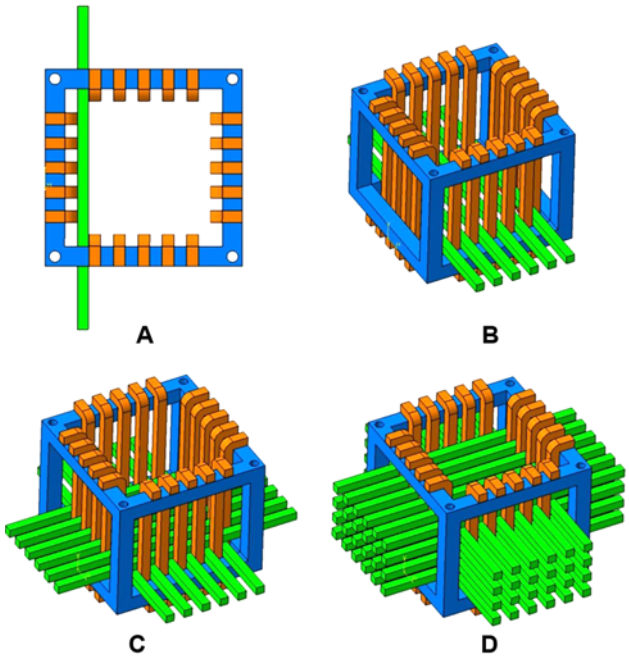

Figure 2. Assembling process of "mold" for wire-network molding technique: (A) and (B) sequential process of assembling "networking wire" into "outer wall," (C) assembling "networking wire" for the second layer, (D) fabricated "open mold" for WNM (green-colored structure: networking wire which is metal wire having rectangular cross-section).

fixed by bending at the end of wires. Wires are positioned with designed interval which is same as the size of used wires. For the regular interval of sidewall frame, grooves are manufactured at the designed positions of the base frame. Eventually, after positioning metal wires, the outer wall is manufactured such as Fig 1D. At Fig 2, same metal wires are depicted as green-colored columns to be distinguished clearly from the side wall usage of Fig 1. As Fig 2, metal wires depicted as green-colored columns are used as network configuration. Wires were inserted into the interval between sidewall wires as Fig 2A and B. According to this procedure, one layer with metal wires is assembled as Fig 2B. After that, as Fig 2C, wires are inserted into the interval between sidewall wires in the different direction, which is 90 degrees rotated. By repeating this procedure, we piled up layer by layer upto the designed height. Lastly, to enclose the top and bottom of mold, top component with inlet and bottom component are assembled as Fig 3. Top and bottom component are also manufactured by Rapid Prototyping machine (Invision HR®). To ensure the sealing between top, bottom components and outer wall, fixation by bolts and nuts is used as Fig 3.

2.2.2 Feasibility Test for the Fabrication of 3D Scaffold with Alginate Gel using WNM Technique

A prototype mold was prepared as depicted in Fig 4A based on the design of Fig 3. Prepared 2 wt% alginate solution was injected into the mold by syringe as depicted in Fig 4B. After

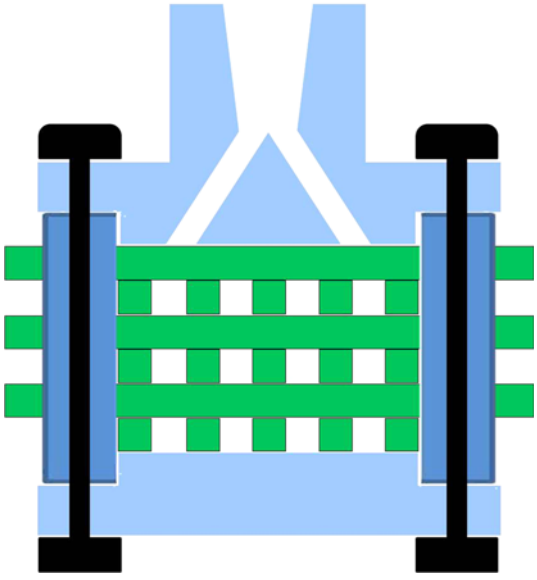

Figure 3. Cross-section view of assembled "mold" with an inlet and bottom components (upper light-blue-colored structure: inlet component, lower light-blue-colored structure: bottom component, black-colored structure: clamping components with bolts and nuts).

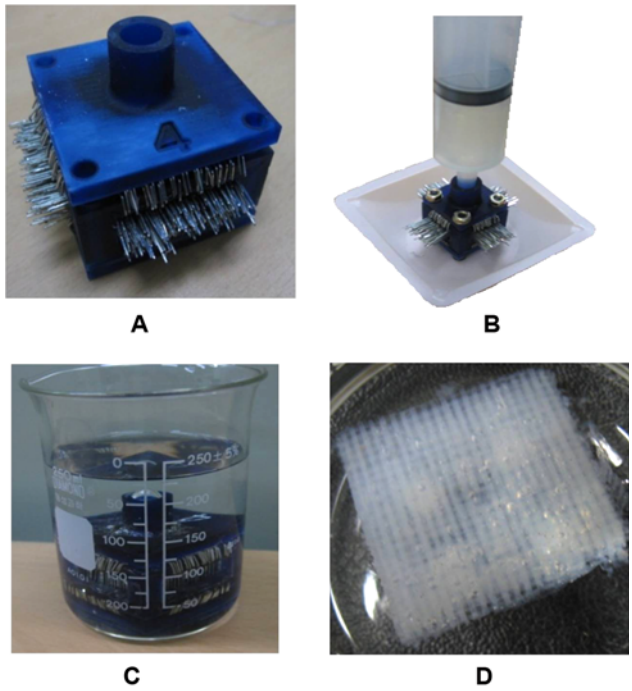

Figure 4. Fabrication process of alginate scaffold using wire-network molding technique: (A) a prepared mold, (B) injection of 2 wt% alginate gel by syringe, (C) soaking in 5 wt% $CaCl_2$ solution for 5 min, (D) fabricated 2 wt% alginate scaffold with interconnected pore.

that, for the gelation of alginate, the mold was soaked in 5 wt% $CaCl_2$ solution for 5 min such as Fig 4C. Afterward, three dimensional alginate scaffold with interconnected pores could be fabricated as Fig 4D by removing the wires and mold after taking out the mold from $CaCl_2$ solution. For the feasibility of fabrication method, wires with 1000 μm thickness are used and the inner size of mold is 29 mm×29 mm×5 mm. Also, we have tested the possibility of fabrication with different concentration of alginate using 1.5 and 4 wt% alginate gel as Fig 5. Fig 5A and 5B are scaffolds fabricated using 1.5 wt% and 4 wt% alginate, respectively. Through this feasibility test of Fig 5, we clarified the fabrication range of alginate concentration is from 1.5 wt% to 4 wt% using our technique to fabricate 3D alginate scaffold with interconnected pores. Obviously, with over 4 wt% concentration alginate gel, we could fabricate 3D scaffold because higher concentration of alginate gives higher stiffness after gelation. In this study, we have used 2 wt% concentration of alginate gel to fabricate scaffold, because too much high concentration of alginate could lead to bad diffusion which could inhibit the delivery of nutrition and waste.

Additionally, to test the feasibility of fabrication with wires with smaller size, we manufactured molds which can fabricate

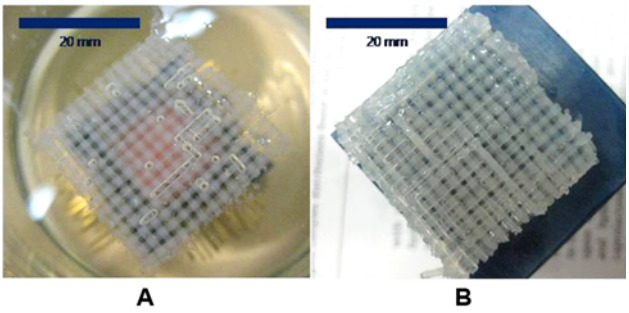

Figure 5. Fabricated alginate scaffolds with pore and strand size of 1000 μm: (A) 1.5 wt% alginate scaffold, (B) 4 wt% alginate scaffold.

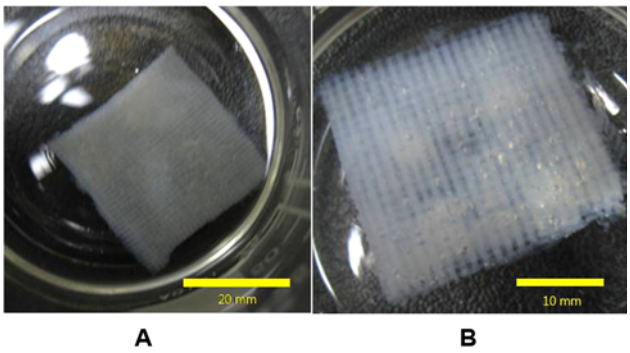

Figure 6. Fabricated 2 wt% alginate scaffolds with different strand sizes: (A) an alginate scaffold with 400 μm strand size and 50% porosity, (B) an alginate scaffold with 600 μm and 50% porosity.

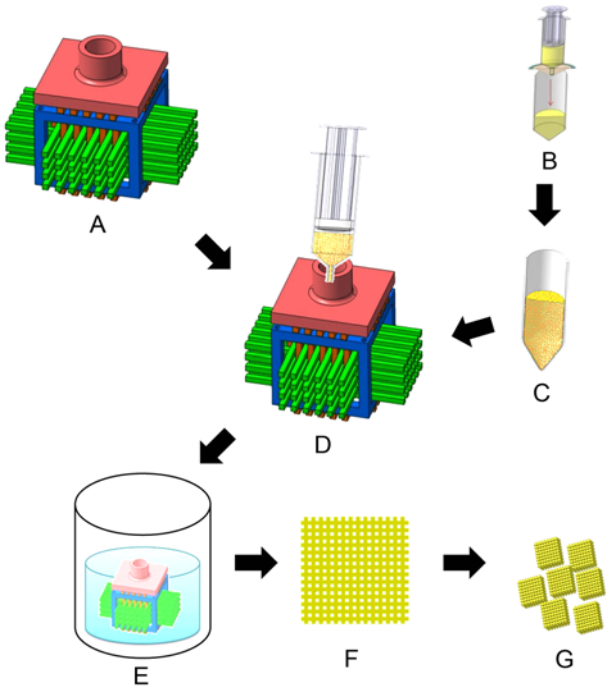

Figure 7. of fabrication of hydrogel scaffold with encapsulated cell: (A) assembled mold, (B) filtering 2% alginate gel, (C) encapsulation of alginate gel and hMSCs cell, (D) inject encapsulated 2% alginate gel into the mold, (E) soak in 5% CaCl2 solution for 5 min, (F) fabricated alginate scaffold sheet with size 29 by 29 by 5 mm after demold, (G) fabricated alginate scaffolds with size 5 by 5 by 5 mm after cutted sheet by scalpel.

scaffold with 29 mm×29 mm×5 mm size using metal wires with the cross section of 400 μm×400 μm and 600 μm×600 μm, respectively. Using these molds, scaffolds with 400 μm and 600 μm pores were fabricated as Fig 6A and 6B, respectively.

As a result, we could fabricate alginate scaffolds with 50% porosity using wires that makes network structure in the mold with the range of alginate concentration from 1.5 wt% to 4 wt% and 400, 600, 1000 μm pore size, respectively.

2.2.3 Sterilization for Experiment

Prior to the experiment, all fabricating components were sterilized by autoclave or ethanol and UV treatment. In this study, the mold, wires, beaker, scalpel, long nose pliers, beaker, and $CaCl_2$ solution are used after sterilization for about 1 hr at 125°C in the autoclave. PDMS mold for making control scaffold without pores, cable ties were sterilized by ethanol and UV treatment. 2 wt% alginate gel was filtered using filter with 0.2 mm pore size as depicted in Fig 7B.

2.2.4 Fabrication of Cell Encapsulated Alginate Scaffold

After completion of sterilization treatment, we fabricated cell-encapsulated scaffold in clean bench as Fig 7. Firstly, the assembled molds having wires with the cross section of 400 μm ×400 μm and 600 μm×600 μm, respectively were prepared after sterilization as Fig 7A. At the same time, 2 wt% alginate gel which is filtered by filter with 0.2 μm pore size was prepared as Fig 7B. At the filtered alginate gel, hMSCs (human Mesenchymal stem cells) are added with 8×10^4 cells/cm3 concentration as Fig 7C. After that, we could mix by pipetting the 1.5% alginate gel and hMSCs. Cell-encapsulated alginate gel was injected into the prepared mold using syringe as Fig 7D. Subsequently, the mold which contained cell-encapsulated alginate gel was gelated by soaking in 5 wt% $CaCl_2$ solution for 5 minutes as depicted in Fig 7E. After taking out the mold from $CaCl_2$ solution, wires are separated from the mold and the mold is removed from alginate gel as shown in Fig 7F. Afterwards, scaffolds with 5 mm×5 mm×5 mm size are fabricated by cutting using a sterilized scalpel as Fig 7G.

2.2.5 Fabrication of Alginate Scaffolds for Control

Prior to *in vitro* experiment, we fabricated 2 wt% alginate

scaffolds without pore for comparison with 2 wt% alginate porous scaffold. After making a PDMS mold which has 25 wells with 5 mm×5 mm×5 mm size, 2 wt% alginate gel, which is hMSC-encapsulated with 8×10^4 cells/cm^3 concentration, was injected into wells by syringe. After soaking in 5% $CaCl_2$ solution for 5minutes, the PDMS mold is removed from alginate gel. Consequently, alginate scaffolds for control were fabricated with 5 mm×5 mm×5 mm size.

2.3 Biocompatibility Assay using hMSCs

2.3.1 hMSCs Culture

The used hMSC (human Mesenchymal stem cell) is Mesenchymal stem cell derived from iliac crest bone marrow which parceled out from Bone Regeneration Research Institute of Wonkwang University. DMEM (Gibco) medium containing 10% fetal bovine serum (FBS, Gibco, Invitrogen, USA) and 1% penicillin-streptomycin (Gibco) is used for the cell culturing with the condition of 5% CO_2 at 37°C.

2.3.2 Encapsulation of hMSCs into Alginate Gel Scaffold

After separating hMSCs from the culture dish using triple Express (Gibco) and centrifugal separation, gathered cells are refloated in alginate solution. This alginate containing hMSCs was injected into the mold for the WNM and control scaffold and gelated with 5 wt% $CaCl_2$ solution with 5 min soaking. Because of the pore existence or not, the encapsulated cell number in a single scaffold is different. For the control scaffold without pore, the encapsulated cell number is about 10,000. On the contrary, for the WNM scaffold with pore, the encapsulated cell number is about 5,000.

2.4 Numerical Analysis using ABAQUS to Predict the Mechanical Property of Scaffold

To predict mechanical property of hydrogel scaffold, we analyzed scaffold model using ABAQUS 6.9-1 program. We have used a unit model of scaffold as a numerical model, because the scaffold using wire-network molding method is composed of regular patterns. As depicted in Fig 9A, the unit FEM model has 33,289 elements and 50,361 nodes. For preventing locking problem and enhancing the accuracy of solution, Quadratic tetrahedral elements are used and the type of element in ABAQUS is C3D10M. The size of FEM model is 0.8 mm×0.8 mm×0.8 mm. Initial boundary conditions are prescribed as follows. Every displacement of x, y, and z direction is fixed as zero at a single node (marked as triangle in Fig 9A). At the other nodes (marked as circle in Fig 9A) on the bottom surface, only z directional displacement is fixed as zero. At every node on the upper surface, displacement in z-axis is prescribed as -0.08 mm to compress the FEM model. The reason why these boundary conditions are chosen is to simulate the uni-axial compression test to analyze the compressive effective stiffness. Young's modulus (E_0) and poisson's ratio are used as 400 MPa and 0.3. To compress the FEM model, displacement in z-axis is prescribed as 0.08 mm.

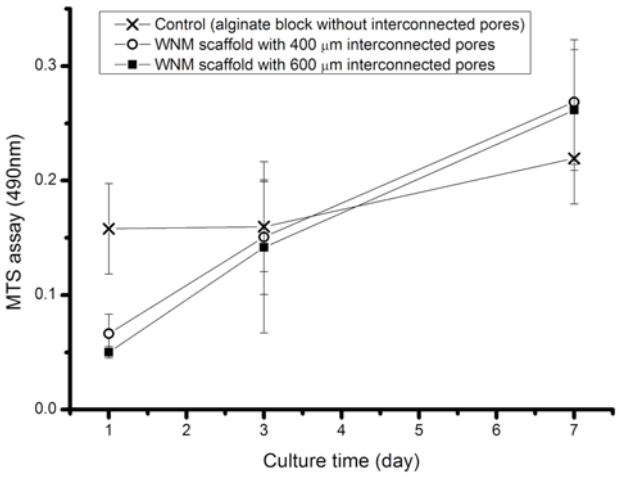

Figure 8. MTS assay at 1, 3 and 7days.

3. Results

3.1 Cell Proliferation Result

As shown in Fig 8, we assessed cell-growth characteristics at 1, 3, and 7 days using MTS assay for the alginate scaffolds fabricated by Wire Network Molding technique compared with control scaffold without pores. As a result, the optical density of control group at 1 days is 0.15±0.039 which is higher than 0.06±0.017 and 0.05±0.005 which are the optical density of 400 μm-WNM scaffold and 600 μm-WNM scaffold, respectively. This result could be explained by the difference of initial amount of encapsulated cell number as mentioned before. The difference of initial amount of cells is caused by the volume difference between WNM scaffold and control. The volume of control scaffold is larger than WNM scaffold, because the control scaffold has no pores. The meaningful results are revealed at 3-day and 7-day MTS assay. The optical density of 400 μm-WNM scaffold and 600 μm-WNM scaffold are clearly more increased than that of control scaffold as depicted in Fig 8. According to the previous study,[16] a certain porous structure in the case of scaffold has an effect for the cell attachment and nutrition supply. Moreover, it is obvious that the porous structure could give more effective diffusion to the encapsulated cells because the diffusion is related with the

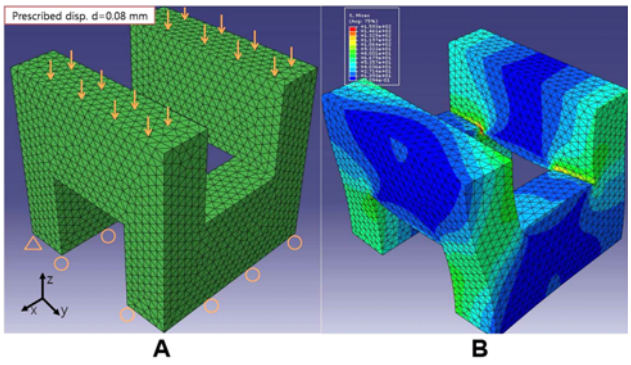

Figure 9. (A) A unit model and boundary condition of scaffold for numerical calculation using ABAQUS, (B) Deformed configuration and von-Mises stress contour after numerical analysis.

distance from the surface. Therefore, 3D cell-encapsulated scaffold with well-interconnected pore could is more effective for the cell growth than the control scaffold without pores.

3.2 Effective Stiffness Result

After numerical calculation using ABAQUS as depicted in Fig 9B, we obtained -8.73634 N as the value of reaction force at the upper surface, which is prescribed with displacement boundary condition of -0.08 mm. After that, to calculate the effective stiffness of unit model, Eqs. (1), (2), and (3) are used.

$$\sigma = E^{eff}\varepsilon \tag{1}$$

$$E^{eff} = \frac{\sigma}{\varepsilon} \tag{2}$$

$$E^{eff} = \frac{P/A}{\delta/L} = \frac{-8.73(N)/0.64(mm^2)}{-0.08(mm)/0.8(mm)} = 136.5(MPa) \tag{3}$$

From Eqs. (1), (2), and (3), the following relations are obtained:

$$E^{eff} \cong 0.34E_0 \tag{4}$$

According to Eq. (4), we could obtain the relation between E_0 and E^{eff} of unit model is 0.34 times. Consequently, WNM scaffold may have the effective stiffness with the value of about 0.34 times as large as the stiffness of the block alginate without interconnected pores.

4. Discussion

In this study, we fabricated 3D alginate scaffold with well-interconnected pore by using a novel method called as 'Wire Network Molding.' For the feasibility test, 1.5 wt%, 2 wt% and 4 wt% alginate gel were used to fabricate 3D porous scaffold with 5 wt% $CaCl_2$ solution during 5 min soaking. Moreover, the wires to make a network which becomes finally interconnected pore are used with the size of 400 μm, 600 μm, and 1000 μm, respectively, for the feasibility of pore-size control. Subsequently, we fabricated hMSC-encapsulated alginate scaffold with 400 μm-WNM and 600 μm-WNM scaffold with 2 wt% alginate gel to assay the cell-growth characteristics. Via MTS assay, the cell-growth characteristics are assessed compared with control alginate scaffold without pore. Consequently, WNM-scaffold were reveals as one of promising scaffold could be used in Tissue Engineering.

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