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氣相高分子支架誘導細胞排列在組織工程上的應用

Vapor-Phase Fabrication of Scaffolds to Achieve Cell Alignment for Tissue Engineering Applications

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本論文係林奕維君(B07504043)在國立臺灣大學化學工程學系完成之學士班學生論文，於民國 111 年 5 月 3 日承下列考試委員審查通過及口試及格，特此證明。

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摘要

細胞的排列對於許多的細胞活性相當地重要，因此需要一個能夠誘導細胞排列生長的細胞養殖系統，當此類細胞壞死或是損壞時，能夠適時地進行修復，像是神經細胞、牙髓細胞等。在這份文章中，我們利用由 polychloro-p-xylylene 此聚合物所製成的具有排列性孔洞的高分子支架，來模擬細胞在體內的排列生長情形。由於有好的生物相容性以及生物穩定性，polychloro-p-xylylene 被廣泛地運用在侵入式的醫療設備當中。這個具有排列性孔洞的高分子支架是透過化學氣相沉積 (chemical vapor deposition, CVD) 的程序所製成。將含有羧甲基纖維素 (carboxymethyl cellulose, CMC) 的水溶液沿著一個特定方向結凍並放入化學氣相沉積的反應室中，在高溫下，欲沉積的聚合物單體與昇華的水氣間就會產生質傳的交互作用並得到產物。後續再利用電子掃描顯微鏡 (SEM) 以及 3D 雷射共軛交顯微鏡 (3D laser confocal microscope) 等儀器進行材料的觀察。本實驗所採用的細胞為小鼠胚胎成纖維細胞 (3T3 cell) 以及人類牙髓幹細胞 (hDPSC)，將所選細胞養殖於材料上後並利用螢光顯微鏡進行細胞觀察，可以明顯觀察到細胞成排列生長，後續再利用細胞活性的測量方法量測細胞於材料上的活性。由於目前尚無醫療方法可以有效地治療蛀牙，因此本技術具有相當大的潛力可以做為蛀牙治療的手段之一，甚至有機會可以在許多的組織工程的應用上利用本技術作為主要的核心技術。

Abstract

Cell alignment is a vital component in various cell activities, therefore, a culture system that could induce cell alignment would be of great importance. In this report, we simulated the oriented cell alignment in the body by culturing the cells on an aligned scaffold made of polychloro-p-xylylene, which is a material that is widely used for implantable medical devices due to its biocompatibility and biostability. The aligned scaffold was fabricated by using chemical vapor deposition (CVD) process. In the process, a water solution containing carboxymethyl cellulose (CMC) was slowly frozen before being inserted into the reaction chamber, where a mass transfer between the deposited monomer and sublimating vapor will occur. The scaffold obtained was then observed with scanning electron microscopy, where the aligned structures were clearly seen. Furthermore, 3T3 cells and human dental pulp stem cells (hDPSCs) were cultured on top of the aligned material and when seen through fluorescence microscope, the alignment of cells were also distinctly observed. This shows that the fabricated polychloro-p-xylylene scaffold could facilitate cell alignment and might make a contribute to the tissue engineering application such as the tooth decay treatment.

Keywords: cell alignment, polychloro-p-xylylene, chemical vapor deposition (CVD), carboxymethyl cellulose (CMC), human dental pulp stem cells (hDPSCs).

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Chapter 1. Introduction

1.1 Study background

Cell alignment is crucial for human beings, such as nerve cells allowing messages to travel through our bodies, muscle cells making human limbs to stretch and bend in many directions, dentin cells allowing teeth to grow healthy. Nevertheless, the technology of the restoration of such aligned cells has not been performed maturely. As a result, a biomaterial scaffold which can induce the cells to grow in an aligned manner is in impendence. In this report, 3T3 cells were first cultured on the aligned polychloro-p-xylylene scaffold to validate whether the scaffold could induce the cells to grow in a specified direction and in a more vigorous way. To further apply this technology to tissue engineering, the dentin cells were chosen as the protagonist of this article, because the medical technology nowadays still cannot treat the tooth decay completely.

1.2 Dental pulp

Dental pulp is a highly vascularized and innervated tissue, which is the only soft tissue in such a highly mineralized tooth structure. It plays an indispensable role in maintaining the perception and homeostasis of the tooth as a living organ (Yang et al., 2016 pulp regeneration, 2019 JDR, Pulp stem cell-mediated, Sui). The pulp is surrounded and protected by a channel porous like hard tissue, that is dentin, the main

tissue that supports the entire tooth structure.

When caries or fracture involves to pulp tissue, root canal treatment may be applied to remove the pulp tissue. However, it will lose remarkable levels of dentin [1]. Also, the loss of proprioception will decrease the feedback of chewing force or caries attack that may further put the tooth in danger. Subsequent restoration or prosthesis may cause further structure loss, rendering the tooth susceptible to fracture from cyclic chewing force or traumatic injuries. The dentin-pulp regeneration will reverse the diseased tooth to its previous more natural state, thus introducing the endodontic treatment or operative dentistry into a new era. As a result, there are many studies devoted dentin-pulp complex regeneration which involve cell-based and cell-free approaches [2].

1.3 Tooth decay treatment

Cell-free approach like using biocompatible/ bioactive capping materials, such as mineral trioxide aggregate (MTA), calcium hydroxide, Hap/TCP, nanocrystalline calcium sulfate or tricalcium silicate (e.g., Biodentine), to treat the damaged pulp tissue is one of the solutions to keep the pulp vitality. However, they just repair the pulp tissue to generate the reparative dentin or a newly mineralized barrier, osteo-like tissue, instead of dentin-pulp regeneration to generate the tubular dentin. The mineral density

of reparative dentin is less than that of tubular dentin [3]. Just like the original healthy dentin (e.g., circumpulpal dentin), the tubular dentin regenerated by tissue regeneration will be rich in tiny dentin tubules. They are responsible for the defense mechanism [4, 5], participate in the responsibility of proprioceptive feedback, and provide considerable structural strength. For using cell-based approaches, they always combined with growth factor and scaffold for guided tissue regeneration [6]. We hope that after partial pulp amputation the induced dental pulp stem cells could differentiate into odontoblasts producing new dentin and capillaries, which would stablish local regeneration along with the required growth factors and appropriate resorbable scaffold. However, pulp-dentin complex regeneration is and should be complicated which remains limitations. Odontoblasts being post-mitotic cells lack of ability to proliferate [7]. In addition, although the secretory activity of odontoblasts promotes reparative dentin formation under certain suitable conditions, poorly organized and mineralized matrix is observed compared with primary and secondary dentin [8]. In order to make dentin-pulp regeneration more widely applicable, it should have the same function as the natural one, with complete and stable vascularity reconstruction; re-established sensory perception and completely re-innervate into pre-dentin and dentin tubules; and generate new tubular dentin integrating to the existing dentin and united together. So far, even when conducted with cell-based approaches, most of them could not generate

real tubular dentin. Recently, oligopeptide (Cpne7-DP) was reported to form the tubular dentin in the animal study, which arrests demineralization process and compensates for dentin defects [9]. However, the experimental material cannot be a designable scaffold to provide a well-organized alignment for differentiated odontoblasts, moreover, further studies would be needed to be conducted to validate the tubular dentin like tissue and interpret the formation mechanism.

1.4 Study purpose

In this study, we presented a designable biomaterial scaffold which would induce the human dental pulp stem cells (hDPSCs) differentiated to odontoblasts and aligned the differentiated odontoblast to generate the well-organized odontoblast process. The fabrications of the proposed scaffold materials were based on a reported vapor sublimation and deposition process [10, 11] called chemical vapor deposition (CVD) which utilized agreed water solutions forming iced templates for sublimation and also exploiting a USP Class VI highly biocompatible poly-p-xylylene as the vapor deposition polymer. The important rationales of using the vapor-fabricated scaffolds in the current study provided (i) incorporation of multiple dopants including functional molecules, growth factor proteins, and live cells with customizable of the compositional ratios in one processing step; (ii) a controllable merit of the proposed process enabled

the control of unsteady-state mass transport (e.g. solidification in iced templates, confined volume system with bi-directional mass transport by sublimation and deposition) [10] that avoided undesirable phase separations of these dopants, and can resulted in administered distributions of these dopants in homogeneous, gradient, and/or hierarchical fashions, [12] (iii) adjustable mechanical properties including pore size, porosity of the final scaffold product, and (iv) we additionally hypothesized the employment of an orientation guiding dopant during the sublimation and deposition process to render an aligned porous structure of the deposited poly-p-xylylene matrix. The overall contributions from (i)-(iv) exhibited a final scaffold construction providing the required combination of both physical and biochemical cues for dentin-pulp regeneration.

Chapter 2. Material and Methods

2.1 Principles of aligned scaffold fabrication

The fabrication of the proposed scaffold system exploited a unique vapor sublimation and deposition process by starting with a prepared iced template from a sublimating solvent (e.g. water molecules) and non-sublimating dopants (e.g. functional molecules, growth factor proteins, and live cells), and a solidification process was performed allowing the control of components compositions and distributions/locations under a unsteady-state or steady-state mass transport of the multiple components in one ice template system [12]. Subsequently, the deposition of polychloro-p-xylylene occurred on such a sublimated iced template formed porous polychloro-p-xylylene network instead of a conventional thin film formation of the same poly-p-xylylene on a solid surface [11] and more importantly, the process ensured the evaporation of water molecules with replaced poly-p-xylylene matrix and with arranged composition and location of the non-sublimated components within the polymer matrix.

The aligned scaffold was produced by including an orientation-guiding component of carboxymethyl cellulose (CMC) and based on the same described fabrication process of vapor sublimation and deposition, and exploited CMC as a non-sublimating dopant that was homogeneously distributed in the prepared iced template. The resultant

scaffold product thus comprised polychloro-p-xylylene matrix and distributed CMC. CMC, which is a cellulose derivative, has a structure of long-stripe rods and before being frozen, the CMC in water solution were originally arranged in a random order. For the CMC to line up in a specified direction, a temperature gradient, i.e. a freezing direction or a growth direction, should be applied when freezing the CMC solution. A possible mechanism, as reported by Lee et al., [13] is based on the progressively forward movement of the ice tip during the ice template fabrication process. Although the solution would be frozen anisotropically under its freezing point, but with a temperature gradient, the ice front would move in a sufficiently slow speed along the temperature gradient orientation for it to apply a momentum which pointed to the freezing direction of the random ordered CMC. If the ice front moves too fast, the CMC would be embedded in the ice template without a direction change. Therefore, with an aligned ice template structure, during the fabrication process consisting of sublimation and deposition, chloro-p-xylylene would be deposited along the aligned CMC, and the resultant polychloro-p-xylylene matrix exhibited an aligned structure. Moreover, as CMC is at micron scale, which is the same as the cell, it would be easier to observe the cell grown on the polychloro-p-xylylene matrix when using CMC as the chosen dopant. The schematic diagram of the fabrication process is shown in Figure 1.

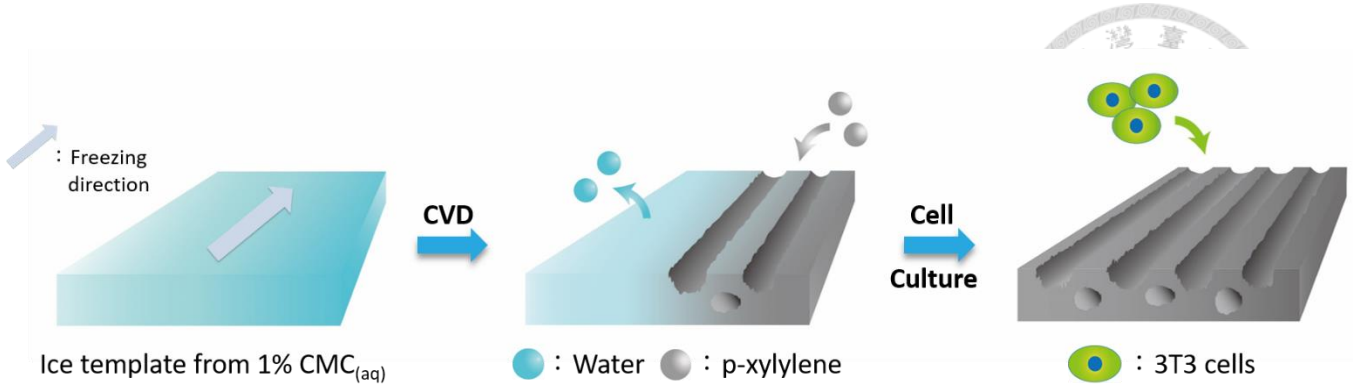

Figure 1. Schematic diagram of the fabrication of porous-aligned polychloro-p-xylylene scaffold for 3T3 cells. The 3T3 cells would then grow aligned the porous structure of the scaffold.

2.2 Scaffold fabrication for hDPSCs

As for the aligned scaffold for hDPSCs, it is quite different from the scaffold used for the 3T3 cells. In the experiment, and based on the fabrication mechanism described above, Wnt-3a and fibroblast growth factor-2 (FGF-2), as well as hDPSCs were prepared in water solution. Non-sublimating components including growth factor and cells were additionally treated in oil-in-water system. Notably, the oil-in-water provided a protection mechanism to ensure the survival of cells during the sublimation and deposition process [14]. Upon the completion of the vapor sublimation and deposition process, an aligned scaffold product composed of polychloro-p-xylylene matrix and distributed Wnt-3a and FGF-2, as well as hDPSCs was produced.

Chapter 3. Experimental Section

3.1 Scaffold fabrication for 3T3 cells

For the preparation of ice templates, 1% CMC solution (w/v) was subjected to a solidification procedure by gradually reduced temperature with the bottom side of the mold attaching to the container which holds the liquid nitrogen. The iced templates were retrieved from the PDMS molds, and subsequently placed in a home-built vapor deposition system for the proposed sublimation and deposition fabrication process [10, [11]. The operating pressure of the CVD system was maintained at approximately 150 mTorr. For the deposition of Parylene, commercial dichloro-[2,2]-paracyclophane (Galxyl C, Galentis, Italy) was used as received. The cyclophane was sublimated at the temperature of approximately 100 °C, and the vaporized cyclophane was then pyrolyzed while passing through a pyrolysis zone held at 670 °C by the transport with a carrier argon gas at the flow rate of 50 sccm, which controlled by a mass flow controller (MFC, MKS Instruments, USA). The deposition rate was adjusted through the at approximately 0.4 Å/s that was monitored by a quartz crystal microbalance (QCM, STM-100/MF, Sycon Instruments, USA).

3.2 Scaffold fabrication for hDPSCs

The bioactive factors, recombinant human Wnt-3a protein (Wnt-3a; R&D systems, USA), recombinant human FGF-2 protein (FGF-2; R&D systems, USA) and carboxymethyl cellulose (CMC; Sigma-Aldrich, USA), were obtained commercially and were stored following the instructions provided by the supplier. Human Dental pulp stem cells (hDPSCs) were isolated from normal human impacted third molars according to the reported method [15]. For the preparation of ice templates, living hDPSCs (approximately 5×10^5 cells in one ice template) in a 50 μL cryoprotectant medium [20.5% w/v dimethyl sulfoxide (Sigma-Aldrich, USA), 15.5% w/v acetamide (Sigma-Aldrich, USA), 10% w/v propylene glycol (Sigma-Aldrich, USA), and 6% w/v polyethylene glycol (PEG, M.W. 8000, Sigma-Aldrich, USA) in MEM-alpha (Thermo Fisher Scientific, USA)] were first mixed with 100 μL glyceryl trioleate oil (Sigma-Aldrich, USA), and the cell-containing oil mixture was then injected (as droplets) into a 250 μL mixture of Wnt-3a (10 or 60 ng/mL), FGF-2 (5 or 10 ng/mL), and CMC (1% w/v)-containing buffer solution that was previously loaded in a polydimethylsiloxane (PDMS) mold [16], and then subjected to a solidification procedure by gradually reduced temperature with the bottom side of the mold attaching to the container which holds the liquid nitrogen. The following procedure is the same as the scaffold fabrication for 3T3 cells.

3.3 Material characterization

SEM images were recorded using an SEM system (NovaTM NanoSEM; FEI, USA), and energy-dispersive X-ray spectroscopy (EDS) was further performed to probe the element analysis. The other images were recorded using an optical microscope (Eclipse 80i; Nikon, Japan) and a 3D laser confocal microscope (VK-9500; Keyence, Japan).

3.4 Stem cell viability and proliferation

Viability (i.e., the percentage of living cells relative to total cells) of the hDPSC-accommodated scaffolds was investigated by staining using a LIVE/DEAD kit (Thermo Fisher Scientific, USA) on days 1, 5, and 21. The cell growth activity was further measured quantitatively using an MTT assay (Sigma-Aldrich, USA). Briefly, the hDPSC-accommodated scaffolds were incubated in the new culture medium containing 10% MTT stock solution (5 mg/mL) for 3 hours at 37 °C. After incubation, the MTT solution was aspirated, and the same volume of dimethyl sulfoxide (DMSO) was added to dissolve the resulting formazan product into a colored solution. The absorbance of the MTT signals was recorded with a microplate reader (BioTek Instruments ELX800,

USA) at a wavelength of 570 nm and the percentage of the increased MTT signals for the corresponding time frames was calculated. To characterize the cell morphology, cultured samples were dried in an oven overnight, sputtered with a conductive layer of platinum, and recorded using a scanning electron microscopy (SEM) system (Nova^{TM} NanoSEM; FEI, USA) with a primary energy of 10 keV and operating pressure of 5 × 10^{-6} Torr. To analyze the cell distribution within the fabricated scaffold, the hDPSCs were first fluorescence-labeled with 2 units/mL of Alexa Fluor® 488-conjugated phalloidin (Thermo Fisher Scientific, USA) to stain the F-actin cytoskeleton and 0.5 μg/mL of 4',6-diamidino-2-phenylindole (DAPI; Thermo Fisher Scientific, USA) to stain the nucleus, and the fluorescence images were captured using a confocal laser scanning microscope (TCS SP5; Leica Microsystems, Germany). Furthermore, the 3D fluorescence images were reconstructed using Imaris (version 9.7).

3.5 Odontoblast differentiation

HDPSCs were cultured in an induction medium and the medium that consisted of MEM alpha in the presence of 10% FBS, 1% antibiotic-antimycotic, 0.1 × 10^{-6} M dexamethasone, 100 μg mL^{-1} L-ascorbic acid (Sigma-Aldrich, USA), and 10 × 10^{-3} M β-glycerol phosphate (Sigma-Aldrich, USA) was renewed twice a week. On day 14, the early-stage odontogenesis of alkaline phosphatase (ALP) activity was determined by

using Fast Red Substrate Kit (Abcam, UK). On day 28, the late-stage odontogenesis of osteocalcin (OCN), and dentin sialophospho-protein (DSPP) expression were confirmed by immunofluorescence staining. Briefly, the hDPSCs and differentiated cells were fixed in 10% formalin for 15 min, washed twice with PBS, and immersed in 0.1% Triton X-100 (Sigma-Aldrich, USA) for 10 min at room temperature, followed by incubating in 1% bovine serum albumin (BSA; Sigma-Aldrich, USA) at room temperature for 30 min. Subsequently, the samples were examined with the corresponding rabbit polyclonal antibody (Abcam, UK) against OCN or DSPP for 1 h, and then stained with Alexa Fluor 488-labeled goat anti-rabbit IgG (Thermo Fisher Scientific, USA) at room temperature for 1 h. A careful rinsing process using PBS buffer (pH = 7.4, Thermo Fisher Scientific, USA) three times was performed during each conjugation and staining procedure. After nuclear staining with 0.5 μg/mL DAPI (Thermo Fisher Scientific, USA) for 10 min, images that captured using a fluorescence microscope with digital camera (Leica Microsystems, Germany) were analyzed using ImageJ software following the reported method to quantify odontogenic activities.

3.6 Alignment analysis

Cell alignment angles were analyzed from recorded SEM and fluorescence images with ImageJ software (NIH, USA).

3.7 Statistical analysis

All the data were expressed as mean values with standard deviation (mean ± SD). Significance was set at the 5% level ($p < 0.05$) using unpaired t-test in GraphPad Prism (version 7.0; USA).

Chapter 4. Results and Discussion

4.1 Characteristics of the 3T3 cells scaffold

Following the vapor sublimation and deposition process, the fabricated aligned polychloro-p-xylylene scaffold was observed under several measuring instruments. As shown in Figure 2. The fabricated aligned scaffold for 3T3 cells is observed with (a) optical microscope, (b) 3D laser confocal microscope, (c) the treatment group under SEM, and (d) the control group under SEM., when observing with optical microscope and 3D laser confocal microscope, it is clear that the pores do align in a specified direction. Furthermore, the pore size is quite uniform rather than irregular configuration. Moreover, for the more detailed observation and reduce the visible error caused by the bright colors, the scaffold was observed under scanning electron microscope (SEM). The difference between the treatment group and the control group is whether the ice template was frozen into a freezing direction. For the treatment group, it did freeze in a temperature gradient, however, the control group was quickly frozen by the -78℃ liquid nitrogen. As could be seen clearly, the pores of the treatment group align considerably evenly, which is an ideal environment for the growing cells that are needed to be aligned. As for the control group, the pores are randomly distributed, which is difficult to induce the cells such as dental cells to grow normally and functionally.

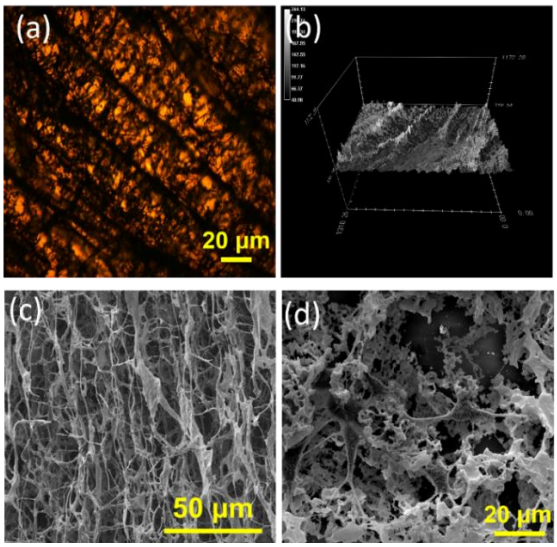

Figure 2. The fabricated aligned scaffold for 3T3 cells is observed with (a) optical microscope, (b) 3D laser confocal microscope, (c) the treatment group under SEM, and (d) the control group under SEM.

4.2 3T3 cells growth behavior on pore-aligned scaffold

3T3 cells are extremely easy to culture, and they usually grow arbitrarily in the environment. The main purpose of culturing 3T3 cells before culturing hDPSCs is to verify that the fabricated biomaterial scaffold can indeed induce the cells which grow arbitrarily to become aligned. 3T3 cells are used to verify that the aligned hDPSCs were induced rather than naturally grew in its own way. As seen in Figure 3, at day 2, a few 3T3 cells grew along the pores for the treatment group and the cells grew arbitrarily for the control group under fluorescence microscope. At day 4, for the treatment group, 3T3 cells almost filled the entire pores, and grew in the specified direction as well. However, for the control group at day 4, there was little change compared to the picture

taken at day 2. Thus, the inducibility of the cell alignment for the polychloro-p-xylylene biomaterial scaffold is verified.

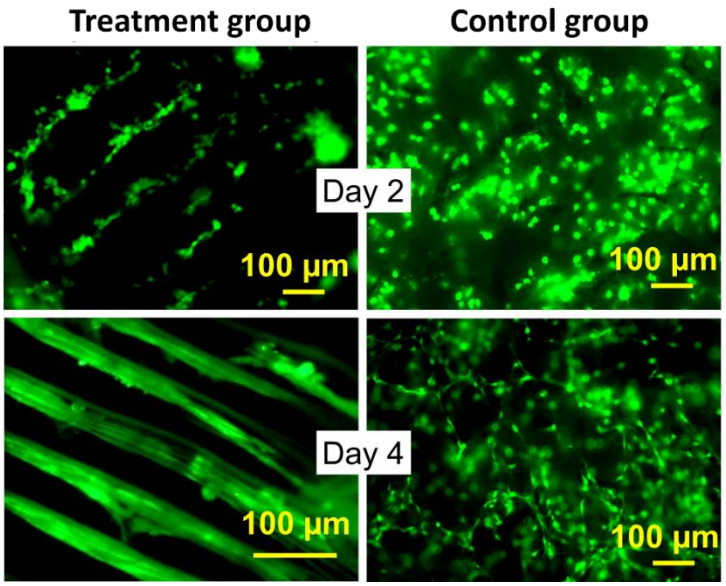

Figure 3. 3T3 cells were cultured on both treatment group and control group and observed at day 2 and day 4 under fluorescence microscope. As seen from the results, the alignment of cells were clearly seen in the treatment group but not in the control group.

4.3 HDPSCs growth behavior on random-pore scaffold

To validate whether growth factor has an impact on the growth behavior of the hDPSCs first, the porous scaffold without porous alignment was used for the validation (the ice template was frozen without freezing direction but with liquid nitrogen). In this report, two combinations of incorporated growth factors are fabricated. First, a biomaterial scaffold with incorporated living hDPSCs, 10 ng/mL Wnt-3a, and 5 ng/mL FGF-2; and a second group of biomaterial scaffold with incorporated living hDPSCs,

60 ng/mL Wnt-3a, and 10 ng/mL FGF-2. A control group, i.e., biomaterial scaffold with only incorporated living hDPSCs but without Wnt3a, and FGF-2, was also fabricated for comparison. At day 5, the cells of the three groups were stained with LIVE/DEAD viability kit and compared. All three groups showed formation of cellular aggregates but in comparison to the control group, both experiment groups showed increased cell metabolic activity, with the second group with the higher concentration of growth factors (60 ng/mL Wnt-3a, and 10 ng/mL FGF-2) having the highest cell metabolic activity among the three groups, thus verifying the successful fabrication of the biomaterial scaffold as well as the growth of the incorporated cells in the scaffold.

Following cell growth in the scaffold, the growth medium was replaced with odontogenic induction medium to induce the odontogenesis of the incorporated cells, and the cells were allowed to differentiate until day 21, where they were again stained with LIVE/DEAD cell viability kit. As shown in Figure 4, the fluorescence intensity shows that the cell metabolic activity of both experiment groups was again much higher in comparison to control group. However, there was only a slight difference in cell metabolic activity between the two experiment groups. Therefore, for the following demonstrations, 10 ng/mL Wnt-3a and 5 ng/mL FGF-2 were chosen for use.

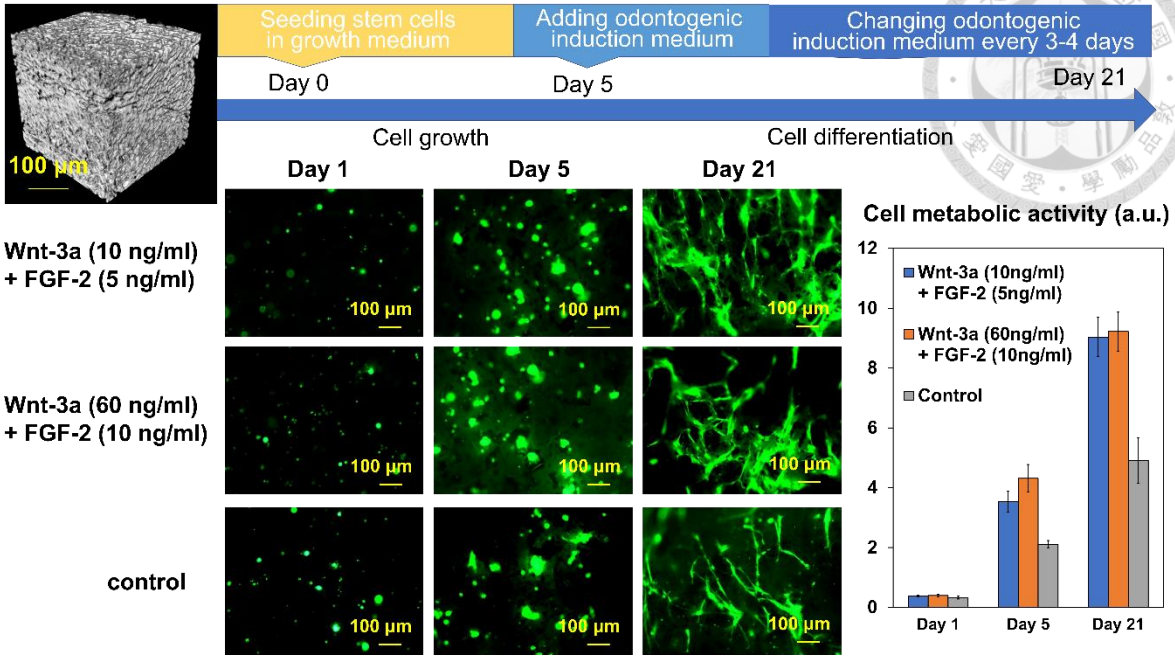

Figure 4. Dental pulp cell culture after porous scaffold process. Following culture for 5 days in growth culture medium, growth of the cells was shown to be enhanced in both scaffolds with encapsulated growth factors in comparison to the control group. After culturing in odontogenic induction medium, cell metabolic activity was also shown to be higher in both experiment groups in comparison to control group.

The odontogenic differentiation of accommodated hDPSCs were examined by measuring the expression of odontogenic markers osteocalcin as well as dentin sialophosphoprotein (DSPP) with immunofluorescence staining. Moreover, the nuclei were also stained with 4',6-diamidino-2-phenylindole (DAPI). As seen in Figure 5, for both OCN and DSPP expression, the fluorescence intensity was higher in the experiment group with incorporated Wnt-3a and FGF-2 than in the control group, showing higher OCN and DSPP expression. This shows that the incorporated growth

factors had helped enhance the odontogenesis, which was a critical requirement for dentin regeneration, in the biomaterial scaffold.

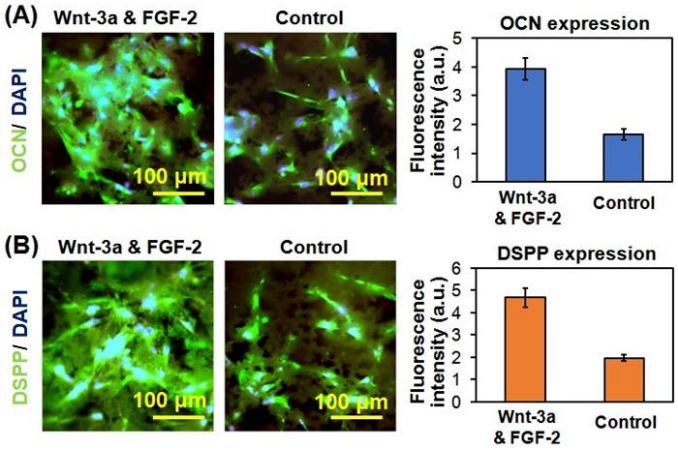

Figure 5. The (A) OCN and (B) DSPP expression of the cells in scaffold with accommodated Wnt-3a and FGF-2, as well as that in the control group were measured after culture in growth medium and odontogenic induction medium for 21 days. The fluorescence intensities measured showed significant higher expression of OCN and DSPP in the accommodated scaffold in comparison to the control group.

4.4 HDPSCs growth behavior on pore-aligned scaffold

To further specify the aligned characterization of the poly-p-xylylene scaffold, the scaffold was observed under the optical microscope, where as seen from Figure 6, the backbone of the CMC were aligned in the same direction parallel to the temperature gradient orientation, which meant that the thin and long pores were aligned. Furthermore, to look into the structure in a larger view, SEM was used to observe the biomaterial scaffold. As seen from Figure 6, the pores were ordered in line with some fibrous roots on the backbone, which was the branched chains of the CMC. In comparison to the control group without CMC, the aligned scaffold has a significantly more aligned structure with a smaller angle range.

Following the successful fabrication of the aligned biomaterial scaffold, hDPSCs were seeded on top of the scaffold to mimic the alignment of odontoblasts at the surface of dental pulp. To observe the seeded cells, the nuclei of the cells were stained with DAPI, and as seen in the results, the seeded cells were shown to have been attached along the fibrous scaffold. Moreover, when the cells were stained with F-actin, similar results were also obtained, where in comparison to the control groups, the cells were shown to follow the direction of the aligned biomaterial scaffold. Cells in the aligned scaffold were shown to be aligned with an angle difference of less than 10° from the freezing direction, while that in control group has an angle range from -90° to 90°.

Therefore, the ability of the biomaterial scaffold in providing an aligned environment similar to natural human body's dentin was verified.

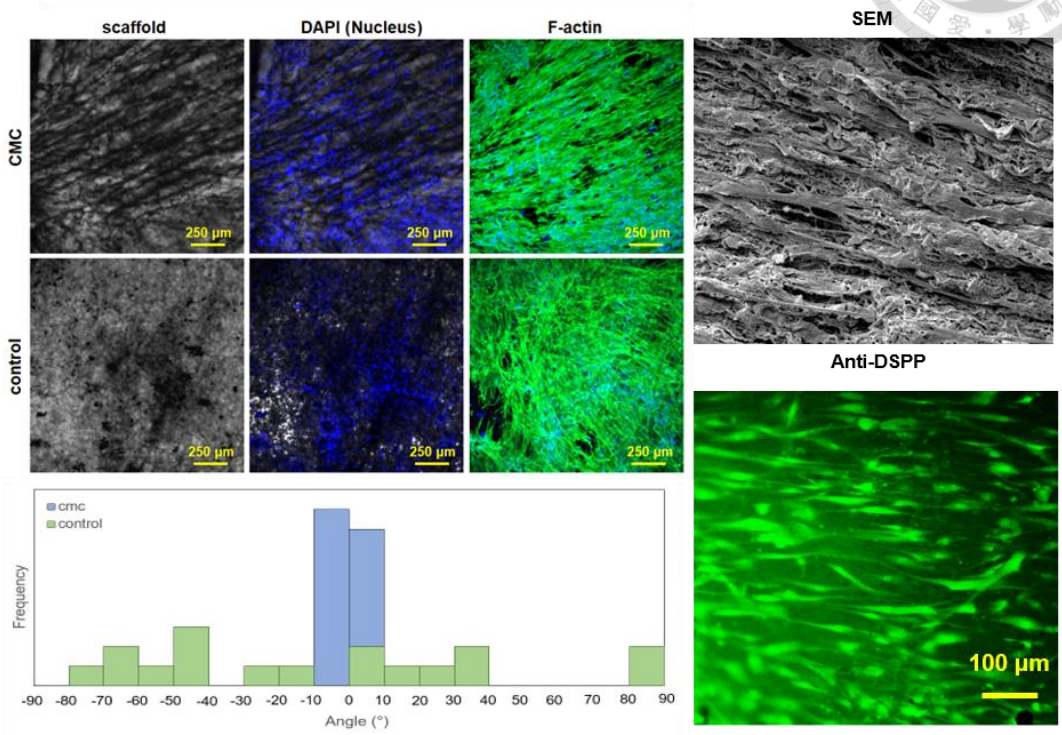

Figure 6. Following culture of the cells in the aligned scaffold, the cells were shown to grow in an aligned matter, in contrast to control group (fabricated without freezing direction), where the cells grow in a random order. SEM images as well as anti-DSPP images also showed the alignment of the encapsulated cells in the scaffold.

Chapter 5. Conclusion

5.1 Recent study conclusion

In this report, a scaffold fabricated from parylene C through chemical vapor deposition process is shown. And the fabricated scaffold has aligned structures as confirmed by scanning electron microscopy results. Following culture of 3T3 cells, the inducibility of the cell alignment for the polychloro-p-xylylene biomaterial scaffold is verified. Moreover, the dental pulp cells were shown to have differentiated to odontoblasts in the porous scaffold. The aligned scaffold was also produced for the alignment of dental pulp cells, which resulted in a more similar environment to the that in the body, where the cells were aligned on the surface of dentin.

5.2 Future work

It is hoped that the technology can be applied to more tissue engineering applications in the future, such as the repairment of the nerve cells, or the regeneration of the muscle cells, which will make a great contribution to the entire field of biomedical and biomaterial engineering.

References

[1] F.F. Demarco, M.S. Rosa, S.B.C. Tarquínio, E. Piva, Influence of the restoration quality on the success of pulpotomy treatment: a preliminary retrospective study, Journal of Applied Oral Science 13(1) (2005) 72-77.

[2] Marwa M. S. Abbass, Aiah A. El-Rashidy, Khadiga M. Sadek, Sara El Moshy, Israa Ahmed Radwan, Dina Rady, Christof E. Dörfer, Karim M. Fawzy El-Sayed, Hydrogels and Dentin-Pulp Complex Regeneration: From the Benchtop to Clinical Translation, Polymers 12 (2020) 2935.

[3] A. Hosoya, H. Nakamura, S. Akahane, K. Yoshiba, N. Yoshiba, T. Ninomiya, K. Hoshi, N. Sahara, E. Kasahara, H. Ozawa, Immunohistochemical Study of Osteodentin in the Unerupted Rat Incisor, Journal of Oral Biosciences 48(2) (2006) 132-137.

[4] D.H. Pashley, The influence of dentin permeability and pulpal blood flow on pulpal solute concentrations, Journal of Endodontics 5(12) (1979) 355-361.

[5] C.-L. Hahn, A.M. Best, The Pulpal Origin of Immunoglobulins in Dentin Beneath Caries: An Immunohistochemical Study, Journal of Endodontics 32(3) (2006) 178-182.

[6] J.P. Vacanti, R. Langer, Tissue engineering: the design and fabrication of living replacement devices for surgical reconstruction and transplantation, The lancet 354 (1999) S32-S34.

[7] V.E. Arana-Chavez, L.F. Massa, Odontoblasts: the cells forming and maintaining dentine, The International Journal of Biochemistry & Cell Biology 36(8) (2004) 1367-1373.

[8] K. Iohara, M. Nakashima, M. Ito, M. Ishikawa, A. Nakasima, A. Akamine, Dentin regeneration by dental pulp stem cell therapy with recombinant human bone morphogenetic protein 2, J Dent Res 83(8) (2004) 590-5.

[9] Y.S. Lee, Y.H. Park, D.S. Lee, Y.M. Seo, J.H. Lee, J.H. Park, H.W. Choung, S.H. Park, W.J. Shon, J.C. Park, Tubular Dentin Regeneration Using a CPNE7-Derived Functional Peptide, Materials (Basel) 13(20) (2020).

[10] H.-Y. Tung, Z.-Y. Guan, T.-Y. Liu, H.-Y. Chen, Vapor sublimation and deposition

to build porous particles and composites, Nature Communications 9(1) (2018) 2564.

[11] H.-Y. Tung, T.-P. Sun, H.-Y. Sun, Z.-Y. Guan, S.-K. Hu, L. Chao, H.-Y. Chen, Construction and control of 3D porous structure based on vapor deposition on sublimation solids, Applied Materials Today 7 (2017) 77-81.

[12] Y.-R. Chiu, Y.-T. Hsu, C.-Y. Wu, T.-H. Lin, Y.-Z. Yang, H.-Y. Chen, Fabrication of Asymmetrical and Gradient Hierarchy Structures of Poly-p-xylylenes on Multiscale Regimes Based on a Vapor-Phase Sublimation and Deposition Process, Chemistry of Materials 32(3) (2020) 1120-1130.

[13] J. Lee, Y. Deng, The morphology and mechanical properties of layer structured cellulose microfibril foams from ice-templating methods, Soft Matter 7(13) (2011) 6034-6040.

[14] H. Feng, L. Wu, A. Xu, B. Hu, T.K. Hei, Z. Yu, Survival of mammalian cells under high vacuum condition for ion bombardment, Cryobiology 49(3) (2004) 241-249.

[15] S. Gronthos, M. Mankani, J. Brahim, P.G. Robey, S. Shi, Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo, Proc Natl Acad Sci U S A 97(25) (2000) 13625-13630.

[16] H.-Y. Chen, J. Lahann, Vapor-Assisted Micropatterning in Replica Structures: A Solventless Approach towards Topologically and Chemically Designable Surfaces, Advanced Materials 19(22) (2007) 3801-3808.