BIOCOMPATIBILITY OF POLYCAPROLACTONE BLENDED HYDROXYAPATITEON THE CULTURE OF HUMAN WHARTON'S JELLY MESENCHYMAL STEM CELL LINE

[1]MONTREE SONJAN, [2]KEWALIN INTHANON, [3]SIRIWADEE CHOMDEJ, [4]WANIDA JANWIKUL, [5]WEERAH WONGKHAM

[1]The Human and Animal Cell Technology Research Unit, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
[2]Kewalin Inthanon, Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Lampang Center, 52190, Thailand
[3]Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand
[4]National Metal and Materials Technology Center, Pathumthani 12120, Thailand
[5]The Human and Animal Cell Technology Research Unit, Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai 50200, Thailand
Email: wwongkham@gmail.com

Abstract: Polycaprolactone/hydroxyapatite (PCL/HA) composites was used in biomedical implantation technologies for decades because of their proper biocompatible properties. Regenerative therapy using stem cell treatment may need special types of biomaterial as initial scaffold. PCL/HA have never been test for biocompatibility to the human Wharton's jelly mesenchymal stem cells, WJMSC. The aim of this work is to study the biocompatible of PCL/HA co-polymer on the in vitro culture of WJMSC. PCL/HA was prepared in 2 platforms; 2D-film and 3D-scaffold. Cell attachment efficacy and cell proliferation assay was investigated by using Enzyme-Linked Immunosorbent Assay (ELISA) through the expression level of focal adhesion kinase (FAK) and the expression of the cell cycle regulator protein, cyclin A, respectively. Result showed that the FAK synthesis of cells on 2D-film was enhanced up at greater than 2-times on those of the 3D-scaffold or polystylene (PS) within 21 days of culture. Cell proliferation on the 2 types of composite polymer was found more than 2-times as much in comparison to those on PS, indicating the cell division enhancement. Differentiation and death of the cells was evaluated by using the real-time PCR techniques on the expression of several groups of genes. Stemness was degraded with time indicating the differentiation of the cells in which verified by the expression of the spontaneous differentiation related genes. Death signals was down regulated with time, showing the biocompatibility of the materials and adaptability of WJMSC to the materials. Results from this preliminary work showed the potential of the PCL/HA composite to be utilized as biomaterial for therapeutic purpose using WJMSC.

Keywords: MSC, FAK, Cyclin A, Polycaprolactone, Hydroxyapatite, Apoptosis, Necrosis, Differentiation.

I. INTRODUCTION

Current therapies in modern medicine commonly involve in preventions, manipulations and controls of diseases through bio-physicochemical principles. Understandings of modified biomaterial interacting on stem cell behaviours have been extensively become as a major part of disease therapies by either direct cell replacement or tissue regeneration. Stem cell-based tissue and organ replacement is still required an accurate and breakthrough research backgrounds to achieve a complete control stem cell fate which not yet clearly understand. Recent review articles discussed how certain types of materials are used as substrates to imitate the physico-chemical microenvironments of cells and tissues (1, 2). Several articles also wisely examined materials that were applied for developmental study of the bone, cartilage, and skin (3-7). Others reported about the combination of multiple pre-differentiated stem cell populations and biomaterials to form hybrid constructions which closely mimicked to the native tissue (8, 9). In this research, we intensively focused on the interaction between biomaterials and stem cells and also a comprehension of the underlying mechanism behind stem cell behaviour and differentiation within their physiological niche.

Stem cells are simply defined as a progeny of cells that have the potential to differentiate into a variety of different lineages. These cells can be isolated from a variety sources including embryos, umbilical cord blood, as well as from adult tissues, as previously reviewed (10). Human umbilical cord, with the unique morphologic properties (11), represents an interesting alternate source for mesenchymal stem cells (MSCs). One important requisite for allogeneic transplantation is low immunogenicity(12). The therapeutic utility of these stem cells can be ascribed to their regenerative and immunomodulatory potential of cells. The Wharton's jelly part of umbilical cord is a special primitive connective layer that acts as a protective tissue for vessels and contains in its stromal compartment cells, called Wharton's jelly MSCs (WJMSCs) (13). Therefore, Wharton's jelly can be an ideal, unique, easily accessible, and noncontroversial source for MSCs. The proliferation and differentiation potency of MSCs derived from human umbilical cord and human bone marrow (BM) is equally identical (14). On the other hand, ultimate large scale therapeutic application of these cells necessitates their maintenance in an undifferentiated

state *in vitro* so that they can be differentiated into specific lineages.

Biomaterials, such as scaffolds or membranes usually be porous, nontoxic, non-immunogenic, biocompatible, adhesive, growth promoting and, in some cases, with biodegradable or resorbableproperty.Degraded products from the transplanted materials also must be non-toxicity, non-immunogenicity and non-clustogenicity. Materials of construction and components should be compatible with the bioprocessing conditions, cleaning solutions and sterilizing conditions. Properties of biocompatible scaffolds, synthetic or natural, that must be taken into careful consideration include optimal fluid transport, delivery of bioactive molecules, material degradation, cell-recognizable surface chemistries, mechanical integrity and the ability to induce signal transduction. Polycaprolactone(PCL) is a biodegradablepolymer with remarkable toughness and good biocompatibility. It is a semi-crystalline aliphatic polymer with sustained biodegradability, good biocompatibility and expectant mechanical strength that has a slower degradation rate and higher fracture energy than most biocompatible polymers (15). However, the low mechanical strength and intrinsic hydrophobic properties of this biodegradable polymer may limit its use in orthopaedics (16). Hydroxyapatite (HA) has been widely investigated as a biocompatible and osteoconductive/inductive biomaterial for use as a porous bone substitute (17). To alter and improve the properties of the material which involves in cell proliferation and differentiation, PCL and HA were blended. The idea of combining bioactive materials and degradable polymers to produce three-dimensional (3D) scaffolds with high porosity is a promising strategy for the design and development of composite systems for hard tissue regeneration materials.

II. MATERIALS AND PROCEDURES

2.1 Polycaprolactone/Hydroxyapatite (PCL/HA) Biopolymer Preparation.

The PCL/HA biopolymer used in this research was displayed in 2 platforms (Figure 1); 2 dimensional (2D) film (PCL/HA-F) and three dimensional (3D) scaffold (PCL/HA-S). Both were obtained from Biomedical Engineering Research Unit, National Metal and Materials Technology Center (MTEC), Pratumtani, Thailand. Processing of copolymer blending and fabrication was carried out by using the standard methodology mentioned elsewhere (18). The mixture ratio of the materials was graded and coded according to the product quality control. The one that we used in this work is coded as MTEC-CMU-P/HA/022556. To prepare the PCL/HA samples for biocompatibility test, both PCL/HA-F and -S were cut into a circular shape of 55 mm in diameter. The circular samples were exposed under ultraviolet light in a biosafety cabinet for 30 minutes in order to sterilization. Next, the samples were placed onto bottom of each well of a 96-well polystylene (PS) culture plate (Nunc®, DK) in which setting up as experimental surfaces (film or scaffold). A set of PS control surfaces was arranged on the well of same plate, but without the PCL/HA samples. Phosphate buffer saline (PBS) was added into either the PS control surface or experimental surface (PCL/HA samples) to avoid samples floating during seeding the cells.

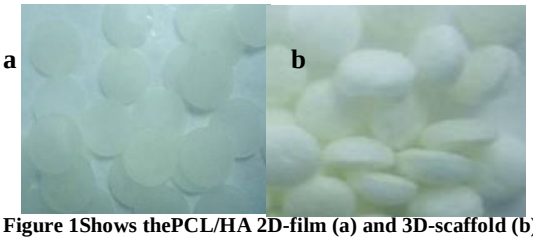


Figure 1Shows thePCL/HA 2D-film (a) and 3D-scaffold (b)

2.2 Cell Culture

BCP-K1, a human Wharton's Jelly mesenchymal stem cell line (WJMSC), was obtained from the Human and Animal Cell Technology Research Laboratory, Department of Biology, Faculty of science, Chiang Mai University, Thailand. The cells were cultured in a completed medium containing alpha-minimal essential medium (α-MEM), supplemented with 10% (v/v) fetal bovine serum (FBS) and 1X antibiotic-antimycotic solution (from 100X stock solution) (all cell culture reagents were purchased from Invitrogen, CA) and maintained at 37°C, 5% CO2 and 95% relative humidity (a standard culture condition) as described in Inthanon et al (2016). The medium was changed every 3 days. Cells were trypsinized by using 0.25% (v/v) trypsin-EDTA (Invitrogen, CA) when reached 70% confluency of a culture surface. Cell viability and density were evaluated by a vital dye, trypan blue, by using a Neubauer-chamber haemocytometer (The Paul Marienfeld GmbH & Co., DE) under a phase-contrast inverted light microscope (Olympus, JP).

2.3 Cellular Responses on PCL/HA biopolymer

The biocompatibility of PCL/HA biopolymer in a form of film and scaffold was evaluated through undifferentiated BCP-K1 (passage <5) to perceive some cytological activities including cell attachment, proliferation, apoptosis or necrosis induction, stemness maintenance and spontaneous differentiation. The detailsof each procedure were separately described below.

I. Cell Attachment Efficiency

Cell attachment assay was evaluated through the expression level of focal adhesion kinase (FAK) protein by using an Enzyme-Linked Immunosorbent Assay (ELISA). To perform the experiment, cell was

prepared as a suspension at a density of 30,000 cell/ml. A 100-ml-cell suspension was seeded onto PS control surface, PCL/HA-F and -S samples which were already placed on the bottom of 96-well plates as mentioned above. The culture plate was then incubated in a standard culture condition. The evaluation of cell attachment was performed after cell seeding for the 1, 3, 5, 9, 12, 15, 18 and 21 day of the cultures. The amount of FAK expression level was examined by following a modified procedure as described previously (Inthanon, et al). Briefly, the attached cells on PCL/HA samples were fixed in 4% (v/v) paraformaldehyde for 30 minutes at room temperature, then washed with gentle shaking by washing buffer (0.05% (v/v) Tween20 in 1X PBS) (Sigma-Aldrich, DE) for 3 times, 5 minutes each. After that, the samples were immersed under quenching buffer (1% (v/v) H_2O_2 and 0.1% (v/v) NaN_3 in 1X PBS) (Sigma-Aldrich, MO) for 20 minutes at room temperature. The samples were washed twice by washing buffer, then replaced the buffer with the pre-warm antibody blocking buffer (5% (v/v) bovine serum albumin (BSA)) (Invitrogen, CA) and incubated for an hour at room temperature. Next, the samples were washed twice with washing buffer and incubated in the diluted anti-FAK primary antibody (1:500 mouse monoclonal antibody) (Invitrogen, CA) at 4°C for overnight.

Washing the samples was performed 3 times in the next morning. The horseradish peroxidase (HRP) labelled secondary antibody (1:2000) (Invitrogen, Carlsbad, CA) was added onto the samples then left for an hour at room temperature. During the waiting times, an alkaline phosphatase substrate for HRP was prepared as mentioned in a manufacture's protocol. Briefly, a tablet of o-phenylenediamine (OPD) (Invitrogen, USA) was dissolved in 12 ml of citrate-phosphate buffer (pH 5.0) containing 0.03% (v/v) hydrogen peroxide. After an hour, the freshly prepared substrate was added onto the samples and incubated at room temperature for 15 minutes in the dark. Finally, the HRP-substrate reaction was stopped by adding 3 M H_2SO_4 onto the samples. The expression of FAK protein was presented as optical density values measuring by a microplate spectrophotometer at OD_{492}. All evaluated samples of each day were done in triplicates.

II. Cell Proliferation Assay

Cyclin A protein was selected as a candidate marker of cell proliferation. Evaluation of protein expression level was performed by ELISA as described in cell attachment assay but using primary the anti-cyclin A antibody (1:500 mouse monoclonal antibody) (Sigma-Aldrich, MO) instead of anti-FAK. Cell seeding density, PCL/HA samples, experimental time and procedures were identical to FAK assay.

III. Transcriptional Responses of Cellular Activities

To get a better understanding on how PCL/HA biopolymers with different platforms effecting on the cells, cellular responses at the transcriptional mRNA expression are important clues revealing the dynamic cell interaction on biopolymers. In this research, three groups of gene associating to cellular activities were assessed by real-time PCR. Lists of genes and primers as mentioned here. (1) *Stemness maintenance related genes*; *POU5F1* (Fw: 5'-ACT GTG TCC CAG GCT TCT TT-3', Rv: 5'-CCA TTT TGG TAC CCC AGG CT-3') and *SSEA4* (Fw: 5'-GCC CTA GAA CTC CAA TCA CA-3', Rv: 5'-CCC AGA TGG TAT TGG ACA CA-3'). (2) *Apoptosis-necrosis related genes*; *Caspase 8* (Fw: 5'-TGC AGG GGC TTT GAC CAC GA-3', Rv: 5'-TGG GGG CCT CCT GTC CAT CA-3'), *Apaf1* (Fw: 5'-TGG CCA GTG CCA AGA TGC ACA-3', Rv: 5'-CGA CCT CCT GCT TGG CCT GC-3') *Bcl2* (Fw: 5'-TGT GGC CTT CTT TGA GTT CG-3', reverse: 5'-TCA CTT GTG GCT CAG ATA GG-3') and *PARP1* (Fw: 5'-AAT CTC CAG GGG GTA GAA CT-3', Rv: 5'-CAG AGC CTG TTG AAG TTG TG-3'). And (3) *spontaneous differentiation related genes*; *Nestin* (Fw: 5'-TCC TGC TCG CTC TCT ACT TT-3', Rv: 5'-GGA AGA GGT GAT GGA ACC AC-3'), *PPARγ 2* (Fw: 5'-GCA TTA TGA GAC ATC CCC ACT GC-3', Rv: 5'-CCT ATT GAC CCA GAA AGC GAT TC-3') and *COL2A1* (Fw: 5' –GCT CCC AGA ACA TCA CCT ACC-3', Rv: 5'-TGA ACC TGC TAT TGC CCT CT-3'). *GAPDH* was chosen as a housekeeping gene (Fw: 5'-GCT CCC AGA ACA TCA CCT ACC-3', Rv: 5'-TGA ACC TGC TAT TGC CCT CT-3').

BCP-K1 cells were prepared and cultured on the PCL/HA–F and –S as described in attachment assay. Cells were harvested for total RNA extraction from the cultures of 11 and 21 day by using NucleoSpin® RNA isolation kit (Macherey-Nagel GmbH & Co., KG, DE). Then the mRNA was synthesized to cDNA using ReverTra Ace® qPCR RT master mix (Toyobo, JP). Quantitative real-time PCR was conducted on SensiFASTTM SYBER® No-ROX Kit (Bioline, AU) in EcoTM Real-Time PCR System (Illumina, USA). PCR mixtures were arranged prior to initiate a chain reaction in a denaturation step at 94 °C for 3 minutes followed by 40 cycles of annealing step, at 94 °C for 40 second, 60 °C for 30 second and then at 72 °C for 30 second. Ct values of each gene was normalized by GAPDH and calculated to a relative expression fold in comparison to PS control by using the $2^{-\Delta\Delta CT}$ method as described in Livak and Schmittgen (2001). The PCR reactions were done in three replicates.

2.4 Statistical analysis

All data was represented in mean ± standard deviation (mean ± SD). Statistical analysis was performed using student t-test followed by multiple

comparison method. At the value of $p \leq 0.05$ was considered as a significant difference.

III. RESULTS AND DISCUSSION

3.1 Cell Attachment Efficacy

Cell attachment efficacy on PCL/HA-F and –S was evaluated and presented as the expression level of FAK protein marker(Figure 2). The results revealed that WJMSC on PCL/HA-F significantly expressed the highest amount, of FAK protein at all-time intervals. Gradually increase of FAK expression was also observed on PCL/HA-F until 21 day of the experiment. The cells on PS displayed slightly more FAK expression than PCL-HA scaffold at all-time with no significant difference.

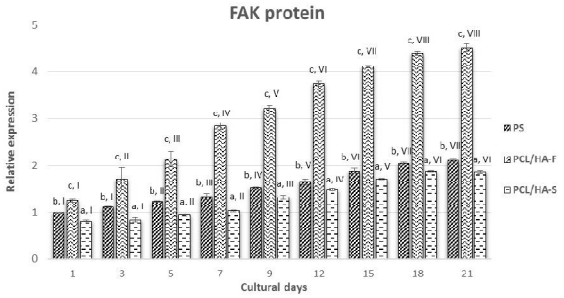


Figure 2 Relative expression of FAK protein from the culture of PCL/HA-F and PCL/HA-S examined by ELISA in comparison to the first day of culture on PS. All data was displayed as mean ± SD. The small case letters (a, b, c) and the Roman number I to VIII represented a significant difference among the samples within each day and among culture days within each sample, respectively.

Cell preferred attached on 2D-film (PCL/HA-F) more than 3D-scaffold (PCL/HA-S) may be because of the interaction between cell membrane and the extracellular matrix (ECM). The polarized membrane-integrin binding that contacts to the surrounding media and surface molecules of the materials and influenced cell attachment and migration. In contrast, the 3D-scaffold have topology that better mimics the architecture formed and therefore cells did not migrate around in scaffold (19). Furthermore, the migration of BCP-K1 have been observed in this experiment on the surface of the 2D-film using SEM (data not shown).

3.2 Cell Proliferation Assay

The ability of cellular proliferation was indicated by the expression level of cyclin A, a cell cycle regulator protein. The results showed that both PCL/HA-F and -S cultures exhibited the higher cyclin A expression than the PS ones and continually increased until the 21 day of experiment (Figure 3). However, the PCL/HA-F cultures significantly showed the highest level of cyclin A among the other samples from day 3 onward.

Similar proliferative support of BCP-K1 on 2D-film and 3D-scaffold may be because of the inspirations of some types of functional molecules on the surface of material providing a better enhancement for cell division. These may also be the influences by the special functional groups due to the presence of HA, a known osteo-conductive material, and the marked improvement in the hydrophilicity of the membrane (20).

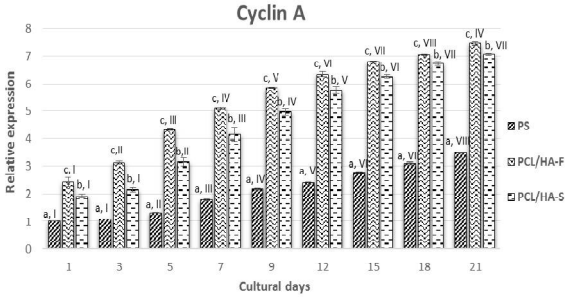


Figure 3 Relative expression of cyclin A from the culture of PCL/HA-F and PCL/HA-S examined by ELISA in comparison to the first day culture of PS. All data was displayed as mean ± SD. The small case letters (a, b, c) and the Roman number I to VIII represented a significant difference among the samples within each day and among culture days within each sample, respectively.

3.3 *Transcriptional Responses of Cellular Activities*

I. Stemness maintenance related genes

The maintenance of stemness in BCP-K1 was evaluated by the expression of the stem cell markers, *POU5F1* and *SSEA4*. The results revealed that the two marker were detectable in all samples on 11-day culture but only *POU5F1* totally absented on 21-day culture (Figure 4a). Either PCL/HA-F or –S cultures expressed lesser both of the 2 markers in comparison to PS. The expression of *SSEA4* from all samples was not differed on 11-day culture, but on 21-days (Figure 4b). Noticeably, PCL/HA-F culture expressed the least *SSEA4* on day 21.

II. Apoptosis and Necrosis related genes

Viability and survival of the cells are important keys for biopolymer development in tissue engineering. From the results, *caspase8* and *Apaf1* were found the highest on PS then PCL/HA-F and PCL/HA-S on day 11, but increased on day 21 with similar level to that of day 11 (Figure 4c, d). In contrast, *Bcl2* was found the highest on day 11 and decreased on day 21 where PLC/HA-S > PS > PLC/HA-F (Figure 4e). For necrosis related gene, *PARP1*, which was detected only in PS culture on day 11 (Figure 4f).

III. Spontaneous differentiation related genes

Nestin was detected on day 11 with the highest level on PS and with equivalent level on PCL/HA-F and PCL/HA-S (Figure 4g). Other genes in this group, *PPARγ 2* and *COL2A1* were undetectable (data not shown).

In general consideration, stem cell fate and differentiation was influenced by the cell-matrix interaction, similar to what happened on the extracellular matrix, by the nanoscale features, topography, and microstructure (21). The PCL/HA materials in this study exhibited a rather unclear mechanism of stemness maintenance due to the

contradicted expression of POU5F1 and SSEA4. The more survival of cells on both 2D and 3Dplatforms, observed by the expression of caspase8 and Apaf1 in comparison to PS, was obviously a pro- to the material. Moreover, this material exhibited a non-serious inductive activities of any kind of cell death. The only detectable potential of inductive differentiation ofPCL/HA in this study was the neuronal differentiation by up the regulation of Nestin. Many kinds of scaffold directly stimulate stem cell and progenitor cell differentiation to neurons and promote and guide neurite outgrowth (22). Even though the cells expressed Nestin and decreased stemness genes expressions, the differentiation patterns was still unclear.

In this experiment, however, differentiation lineage of other types including adipocyte and chondrocyte was not found. In short, this novel copolymer, PCL/HA, potentially maintained WJMSC stemness and also initiated neuronal differentiation. These effects were assumed to be due to interactions between the material properties and the cells, which will be useful for the advancement of tissue engineering for neuronal related applications.

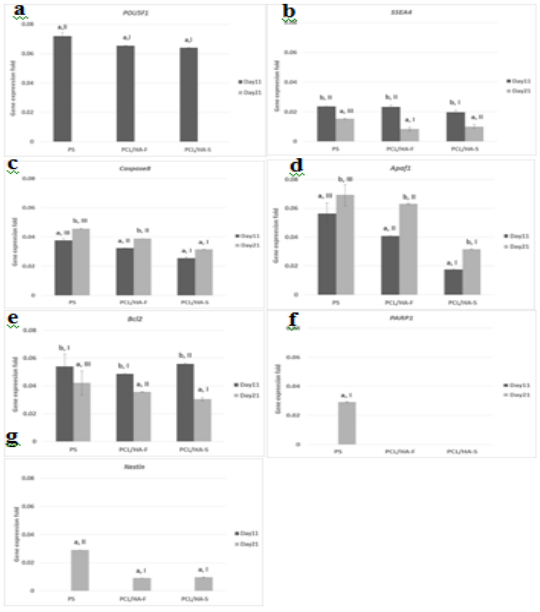


Figure 4 Expression of three groups of genes; (1) Stemness maintenance related genes, *POU5F1* (a) and *SSEA4* (b); (2) Apoptosis and necrosis related genes, *caspase8* (c), *Apaf1* (d), *Bcl2* (e) and *PARP1* (f);(3)Spontaneousdifferentiation related genes; *Nestin* (g). All data was displayed as mean ± SD. The small case letters; a, b represented a significant difference between day 11 and 21 on each sample. The Roman numbers; I, II, III represented a significant difference among the samples on the same day.

CONCLUSIONS

This study revealed the biocompatible properties of PCL/HA composite polymer using the high potential of therapeutic WJMSCs. At the initial stage of culture during the 21 days of investigation, cells were gradually decreased the stemness and induced into neuronal lineage. Differentiation towards the neuronal lineage was initiated by the polymer topography. This material did not stimulate chondrocytes or adipocytes differentiation. The polymer enhanced cell proliferation, cell survival and protected the cells from being prevailed to death. Therefore, this study showed that PCL/HA might be hypothetically useful additions to medical stem cell therapy and tissue engineering technologies.

ACKNOWLEDGMENTS

This work was financially supported by the Thailand Graduate Institute of Science and Technology Scholarship (TGIST). Molecular reagent and PCL/HA membranewas provided byNational Metal and Materials Technology Center, Pathumthani, Thailand.

REFERENCES

[1] P.K. Yarlagadda, M. Chandrasekharan, J.Y. Shyan, Recent advances and current developments in tissue scaffolding, Biomed. Mater. Eng., 15, 159–177. 2005.
[2] T. Ahsan, R.M. Nerem, Bioengineered tissues: the science, the technology, and the industry, Orthod. Craniofac. Res. 8, 134–140, 2005.
[3] A. El-Ghannam, Bone reconstruction: from bioceramics to tissue engineering, Expert. Rev. Med. Devices 2, 87–101, 2005.
[4] R.E. Horch, J. Kopp, U. Kneser, J. Beier, A.D. Bach, Tissue engineering of cultured skin substitutes, J. Cell. Mol. Med. 9, 592–608, 2005.
[5] J.Y. Lim, H.J. Donahue, Biomaterial characteristics important to skeletal tissue engineering, J. Musculoskelet. Neuronal. Interact. 4, 396–398, 2004.
[6] J. Raghunath, H.J. Salacinski, K.M. Sales, P.E. Butler, A.M. Seifalian, Advancing cartilage tissue engineering: the application of stem cell technology, Curr. Opin. Biotechnol. 16, 503–509. 2005.
[7] H. Yoshikawa, A. Myoui, Bone tissue engineering with porous hydroxyapatite ceramics, J. Artif. Organs 8, 131–136, 2005
[8] J. Elisseeff, C. Puleo, F. Yang, B. Sharma, Advances in skeletal tissue engineering with hydrogels, Orthod. Craniofac. Res.150–161, 2005.
[9] M.N. Rahaman, J.J. Mao, Stem cell-based composite tissue constructs for regenerative medicine, Biotechnol. Bioeng. 91, 261–284, 2005.
[10] M. Mimeault, S.K. Batra, Concise review: recent advances on the significance of stem cells in tissue regeneration and cancer therapies, Stem Cells. 24, 2319–2345, 2006
[11] Sakamoto T, Ono H, Saito Y. Electron microscopic histochemical studies on the localization of hyaluronic acid in Wharton's jelly of the human umbilical cord [in Japanese]. Nippon Sanka Fujinka Gakkai Zasshi 1996; 48:501– 507.
[12] Prasanna, S.J.; Jahnavi, V.S. Wharton's jelly mesenchymal stem cells as off-the -shelf cellular therapeutics: A closer look into their regenerative and immunomodulatory properties. Open Tissue Eng. Regen. Med. J. 4, 28–38, 2011.
[13] McElreavey KD, Irvine AI, Ennis KT et al. Isolation, culture and characterisation of fibroblast-like cells derived from the Wharton's jelly portion of human umbilical cord. Biochem Soc Trans. 19: 29S, 1991.

[14] Deuse, T.; Stubbendorff, M.; Tang-Quan, K.; Phillips, N.; Kay, M.A.; Eiermann, T.; Phan, T.T.; Volk, H.D.; Reichenspurner, H.; Robbins, R.C.; et al. Immunogenicity and immunomodulatory properties of umbilical cord lining mesenchymal stem cells. Cell Transplant. 20, 655–667, 2011.

[15] A.P.D. Elfick, Biomaterials. 23,446–447, 2002.

[16] J.A. Roether, A.R. Boccaccini, L.L. Hench, V. Maquet, S. Gautier, R. Jerome, Biomaterials. 23, 3871–3878, 2002.

[17] S.J. Hollister, Porous scaffold design for tissue engineering. Nat Mater. 4:518–524, 2005.

[18] P. Uppanan, B. Thavornyutikarn, W. Kosorn, P. Kaewkong, W, Janvikul, Enhancement of chondrocyte proliferation, distribution, and functions within polycaprolactone scaffolds by surface treatments. Journal of Biomedical Materials
Research Part A. 103, 7, 2322–2332, 2014

[19] R.S. Gieni, M.J. Hendzel, 2008. Mechanotransduction from the ECM to the genome: Are the pieces now in place? J Cell Biochem 104(6):1964–1987.

[20] Concepcio ´n Serrano M, Pagani R, Manzano M, Comas JV, Teresa Portole ´s M. Mitochondrial membrane potential and reactive oxygen species content of endothelial and smooth muscle cells cultured on poly (e-caprolactone) films. Biomaterials.
27: 4706-4714, 2006.

[21] Z. Zhang, M. Rouabhia, Z. Wang et al., "Electrically conductive biodegradable polymer composite for nerve regeneration: electricity-stimulated neurite outgrowth and axon regeneration," Artificial Organs, vol. 31, no. 1, pp. 13–22, 2007.

[22] S. Tunma, K. Inthanon, C. Chaiwong, J. Pumchusak, W. Wongkham, and D. Boonyawan, "Improving the attachment and proliferation of umbilical cord mesenchymal stem cells on modified polystyrene by nitrogen-containing plasma," Cytotechnology, vol. 65, no. 1, pp. 119–134, 2013.

★★★