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論文 / 著書情報
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Title(English)	Synthesis of Hyperbranched Polysiloxysilane Hybrid Copolymer: Convenient Linker Toward Easy Surface Modification, and Application to Thermoresponsive Surface for Cell Cultivation
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（博士課程）
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論 文 要 旨

THESIS SUMMARY

専攻： Department of	Organic and Polymeric Materials 専攻	申請学位（専攻分野）： Academic Degree Requested	博士 (Engineering) Doctor of
学生氏名： Student's Name	GILLET RENAUD THOMAS GIM ARES EROS	指導教員（主）： Academic Advisor(main)	Professor Masa-aki Kakimoto
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要旨（英文 800 語程度）
Thesis Summary (approx.800 English Words)

Cell sheet cultivation is an important topic of research regarding the regenerative medicine since no donors are required reducing greatly the possibility of rejection upon transplantation. Current method of cell sheet cultivation is lacking an easy way to remove the sheet from the cultivation support without damaging it. In order to facilitate the removal of the sheet, recently, thermo-responsive cell culture surface has been developed using poly(*N*-isopropylacrylamide) (PNIPAM). Due to the drastic change in the hydrophilicity of PNIPAM, which exhibits a lower critical solution temperature (LCST) of 32° C, it is possible to change the surface hydrophilicity by simply changing the temperature. Above the LCST, the hydrophobic surface permits the cell cultivation, and by reducing the temperature below the LCST, the cell sheets spontaneously detach from the surface. However, the introduction of PNIPAM onto the surface is done by electron beam polymerization, which requires complicated equipment. Recently, our group design similar system using hyperbranched polystyrene, however, such system is limited to polystyrene (PSt) dish. In the current work, we present an easy and convenient strategy to modify the surface with PNIPAM by drop casting hyperbranched polysiloxysilane block poly(*N*-isopropylacrylamide) (HBPSi-*b*-PNIPAM). The main goal of this work is to obtain a system which can be introduced onto various surfaces.

Hyperbranched polysiloxysilane vinyl-terminated was obtained by polycondensation of 1,5-divinyl-1,1,3,5,5-pentamethyltrisiloxane (AB_2 monomer type), and vinyl termini were changed to hydroxyl groups. *S*-1-dodecyl-*S*'-(α,α'-dimethyl-α''-acetic acid) trithiocarbonate, a reversible addition-fragmentation chain-transfer (RAFT) polymerization chain transfer agent (CTA) was then introduced at the termini by esterification of the hydroxyl groups in order to further grow PNIPAM by RAFT polymerization in order to synthesize HBPSi-*b*-PNIPAM. RAFT polymerization of NIPAM from CTA-terminated HBPS was carried out at 65° C in presence of AIBN as initiator in THF to give HBPSi-*b*-PNIPAM. The PNIPAM segments were analyzed by cleaving them from HBPS by the hydrolysis of the ester group of CTA using an aqueous NaOH solution.

HBPSi-*b*-PNIPAM solutions in THF:MeOH (1:4, *v*:*v*) were casted onto PSt dishes for cell cultivation. Mouse 3T3 fibroblast cells were seeded on the polymer-coated dish and cultivated in a CO_2 incubator (37 ° C, 5% CO_2). After two or four days, the surface of the culture dish was observed with an optical microscope while the temperature was maintained at 37 ° C. Then, the sample was placed in an incubator (20 ° C, 5% CO_2) for 15 min, and the surface was observed again. After two days of cultivation, HBPSi-*b*-PNIPAM modified PSt dishes showed growth of the cells, and cell sheet could be obtained after 4 days of cultivation. After reducing the temperature to 20° C for 15 minutes, cell sheet could detach from PSt modified surface with relatively short PNIPAM (DP_n comprised between 60 to 190), while the cell sheet could not detach from the modified surface with relatively long PNIPAM (DP_n over 200).

After successful modification of PSt dishes surface with HBPSi-*b*-PNIPAM, glass plate surface modification was attempted. Solutions of HBPSi-*b*-PNIPAM in THF were casted onto piranha pre-treated glass plates, and the solvent was dried in air overnight, follow by heating at 100° C for 24h. In order to remove unattached HBPSi-*b*-PNIPAM, the glass plates were washed with ultrapure water, and then dried in air overnight. Cells were cultivated on unmodified and PNIPAM modified glass plates for four days. After cooling down to 20° C, cell sheet could not detach from unmodified glass plate whereas detachment was achieved on PNIPAM modified glass plate surface. Different copolymers were also tested for the surface modification of glass slides, and their application to cell cultivation. The HBPSi-*b*-PNIPAM modified glass slide was successfully reused to thermoresponsive surface for cell cultivation owing the strong adhesion between HBPSi-*b*-PNIPAM and the glass slide.

Finally, in order to improve the cells growth, a PSt brushes layer was introduced onto the surface of the glass slide before casting HBPSi-*b*-PNIPAM. Cell cultivation on PSt brush glass slide modified with HBPSi-*b*-PNIPAM P3 shown than the smoothness of the surface played a critical role. In the case where the surface was rough, the cell sheet was not able to detach from the surface after reducing the temperature to 20 ° C for 15 minutes. In the case of smoother surface, the cell sheet could be recovered by reducing the temperature to 20 ° C for 15 minutes, however, the cells growth was hindered compared to bare glass slide.

In conclusion, HBPSi-*b*-PNIPAM was successfully synthesized by RAFT polymerization of NIPAM from the CTA's termini of HBPS and different size of PNIPAM were synthesized. Surface of PSt dishes and glass slides could be modified, and cell cultivation was possible and showed efficient thermoresponsiveness to detach the cell sheets from their supports.

備考 ： 論文要旨は、和文 2000 字と英文 300 語を 1 部ずつ提出するか、もしくは英文 800 語を 1 部提出してください。
Note : Thesis Summary should be submitted in either a copy of 2000 Japanese Characters and 300 Words (English) or 1copy of 800 Words (English).

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