Cancer cell attachment on a blood compatible polymer, poly (2-methoxyethyl acrylate) (PMEA)

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Statement of Purpose: Cell attachment has been focused in many scientific fields such as tissue engineering and medicine. There are many researches to develop blood compatible polymers on which blood cells and other types of cells cannot attach. However, there are no blood compatible polymers that allow the attachment of non-blood cells but not blood cells without any specific ligands to our knowledge. Such blood compatible polymers will be useful for blood-contact application such as stem cell isolation from blood and bioartificial tissue construction (*eg*. artificial blood vessel and liver). In the patients of metastatic cancer, cancer cells circulate in the blood as circulating tumor cells (CTCs). The regulation of cancer cell attachment by blood compatible polymers will open the way for a new cancer therapy called as personalized medicine using CTCs in human blood. Recently, we have found that cancer cells but not platelets can attach on blood compatible polymer, poly (2-methoxyethyl acrylate), PMEA and its analogous polymers. In this study, we investigate the attachment mechanism of cancer cells on these polymers, especially focusing on integrin-dependent and –independent mechanisms.

Methods: Breast cancer cell line, MDA-MB-231, and breast benign tumor cell line, MCF-10A were used in this research. These cells were allowed to attach on PMEA, poly (tetrahydrofurfuryl acrylate) (PTHFA), polyethylene terephthalate (PET), and poly (2-methacryloyloxyethyl phosphorylcholine-*co*-buthyl methacrylate) (PMPC). Also, cells were allowed to attach on fibronectin (FN)-coated PET substrate. The attached cells were counted in three randomly selected fields using an optical microscope after crystal violet staining. The exposure degrees of cell attachment site in fibrinogen and fibronectin were detected by ELISA with the site-specific antibodies. The amounts of proteins adsorbed on the substrates from serum-containing medium were measured by μBCA method.

Results: Both cells were started to attach on FN within 10 min. Cell attachment on PMEA occurred more rapidly than those on other polymer substrates. The cells hardly attached within 3 hrs on PMPC. To identify the mechanisms underlying the cell attachment, cell attachment via integrin-mediated mechanism was inhibited using EDTA. MCF-10A attachment was suppressed in the presence of EDTA on all examined substrates completely. Interestingly, MDA-MB-231 attachment on PMEA was not suppressed completely whereas the attachment on other substrates was completely suppressed. Moreover, focal adhesion formation was delayed in MDA-MB-231 on PMEA compared with other substrates. These results suggested that MDA-MB-231 attachment on PMEA via integrin and non-integrin-mediated mechanisms.

As a next step, we try to investigate why cancer cells but not platelets can attach on PMEA and PTHFA. Platelets and cancer cells usually attach on polymer substrates via integrin-dependent interaction to fibrinogen and fibronectin which is adsorbed on the substrates, respectively. Indeed, proteins can be adsorbed on PMEA and PTHFA. The order of protein adsorption was found to be PMPC < PMEA < PTHFA < TCPS. It is possible that fibrinogen and fibronectin are adsorbed on PMEA and PTHFA to expose their cell attachment sites. We examined the exposure degrees of cell attachment sites in fibrinogen and fibronectin to unveil cell attachment mechanism to PMEA and PTHFA. Cell attachment site in fibrinogen was hardly detected on PMEA, PTHFA, and PMPC but not on tissue culture polystyrene (TCPS) which is used instead of PET, suggesting that platelets cannot attach on PMEA, PTHFA, and PMPC. In contrast, cell attachment site in fibronectin was detected on PMEA and PTHFA, similar with TCPS, suggesting that cancer cells can attach on PMEA and PTHFA via integrin-dependent mechanism.

Integrin-independent cell attachment was observed on PMEA in addition to integrin-dependent attachment. Fewer proteins was observed on PMEA than did that on PTHFA and TCPS, suggesting that PMEA substrates was not completely covered with adsorbed proteins to allow cells for the interaction with the substrate directly. We examined cell attachment in serum-free medium to examine whether cells can interact with polymer substrates directly. Cells attached on polymer substrates in serum-free medium more rapidly than did that in serum-containing medium. This result indicates that cells can directly attach on polymer substrates. It seems that MDA-MB-231 attach on PMEA via integrin-dependent mechanism and the direct interaction to polymer substrate (non-integrin mechanism) (Table 1).

Table 1: Summary of the results

Cell type	MDA-MB-231		MCF-10A	
Attachment on FN	Weak		Strong	
Attachment in serum-free medium	Strong		Weak	
Substrate type	PMEA	PTHFA	PMEA	PTHFA
Protein adsorption	Small (Substrate surface was exposed)	Large (Substrate surface was not exposed)	Small (Substrate surface was exposed)	Large (Substrate surface was not exposed)
Cell attachment site exposure	Medium	Large	Medium	Large
Attachment mechanism	Integrin+ Non-Integrin	Integrin	Integrin	Integrin

Conclusion: It is expected that PMEA and PTHFA, which have been newly categorized as new types of blood-compatible polymers, will prove useful for blood-contact biomedical applications such as the isolation of cell from blood and the development of bioartificial tissue under blood contact condition.

References: T Hoshiba. Adv Healthcare Mater. In press.