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Fast and efficient microscale radiosynthesis of 3′-deoxy-3′-[^{18}F]fluorothymidine

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ABSTRACT

Microscale radiosynthesis on a miniaturized device has been reported as a promising tool for positron emission tomography. Here, we studied miscroscale-level radiosynthetic conditions by examining several microscale reactions that affect the radiochemical yield of 3′-deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]FLT). [^{18}F]FLT was prepared with 3-*N*-Boc-5′-*O*-dimethoxytrityl-3′-*O*-nosyl-thymidine using the cartridge purification method. [^{18}F]FLT was prepared with a yield of 71%, and the product's purity was greater than 99%. Our results provide a new guideline for microscale [^{18}F]FLT radiosynthesis.

1. Introduction

Positron emission tomography, or PET for short, detects biomolecules using positron-emitting isotopes. It is a powerful yet non-invasive imaging technique that permits the study of the human body and its illnesses by tracing molecular and biological processes [1]. PET has multiple valuable functions such as metastases detection, drug development, early diagnoses, and biochemical studies. Consequently, the demand for PET has been on the rise, and the role of PET is expected to expand in the field of medicine for purposes of research, ranging from cardiovascular perfusion to tumor monitoring [2].

One of the most widely used radiopharmaceutical for PET imaging is 3′-deoxy-3′-[^{18}F]fluorothymidine ([^{18}F]FLT), a radiolabeled analog of thymidine, which is used for imaging cellular proliferation in vivo [3]. In proliferating cells, phosphorylation of [^{18}F]FLT to ^{18}F-labeled thymidine monophosphate ([^{18}F]FLT-5′-PO_4) is catalyzed by the upregulated enzyme, thymidine kinase-1 (TK). [^{18}F]FLT-5′-PO_4 is accumulated and trapped within the cell by a similar process to that of [^{18}F]FDG-6-PO_4 [4]. It has been discovered that [^{18}F]FLT's accumulation inside proliferating cells correlates to DNA replication. Further studies indicate that the cellular uptake of [^{18}F]FLT has a higher correlation with the rate of cell proliferation in tumors than that of [^{18}F]FDG, a commonly used tumor imaging agent [5]. Moreover, [^{18}F]FLT's resistance to metabolic degradation in vivo and fluorine-18's proper half-life (longer than ^{11}C-labeled thymidine) indicates [^{18}F]FLT's possible advantage to be used as a radiopharmaceutical for tumor imaging [6]. Clinical studies also suggest that [^{18}F]FLT can be a potential tumor imaging agent for assessing several illnesses such as lung cancer and brain tumor [7].

These properties have challenged many researchers to develop novel synthetic methods for synthesizing [^{18}F]FLT, after the method of [^{18}F]FLT radiosynthesis was first introduced in 1991 [8]. Grierson and coworkers outlined a method of synthesizing [^{18}F]FLT starting with the precursor 3-*N*-(2,4-dimethoxybenzyl)-1-[5-*O*-(4,4′-dimethoxytrityl)-3-*O*-nosyl-2-deoxy-β-D-lyxofuranosyl]thymine [9]. However, yield was low and it took a long time for synthesis to complete (100 min). Martine and co-workers developed an automated system for radiosynthesis using 3-*N*-BOC-1-[5-*O*-(4,4′-dimethoxytrityl)-3-*O*-nosyl-2-deoxy-β-D-lyxo-furanosyl]thymine with a higher radiochemical yield [10]. Subsequently, an improved synthetic procedure of 60 min was

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reported by Yun and co-workers [11]. Recently, a simplified and automated method of radiosynthesis using modified PET-MF-2V-IT-I synthesis module was developed [12].

Conventional synthetic methods including synthesis of [^{18}F]FLT have several issues such as cost and facility requirements. In addition, only a small quantity of chemicals is necessary in the process of radiolabelling PET probes. Therefore, it makes sense to develop new technology associated with radiosynthesis and to modify PET radiochemistry to fit miniature systems. Because the amount of [^{18}F]fluoride in the reaction mixture (~50 pmol) is so tiny, and the labeling reaction is bimolecular, significant advantages in the reaction kinetics can be gained by a reduction in the overall solvent volume used to perform the reaction. In addition, the small footprint of these devices means that multiple synthesizers can be placed in a single hot cell, thereby increasing the capacity of radiopharmaceutical laboratories to produce radiotracers without additional investment. As we can see, radiosynthesis of PET probes using microfabrication technology has been shown to be an alternative method for PET tracers, and microfluidic lab-on-chip technologies has been used for synthesizing PET tracers [13]. Recently, we have shown radiochemical reactions for a variety of PET tracers on an electrowetting-on-dielectric-chip, or EWOD for short [14].

Microfluidic synthesis platforms provide key advantages over current PET radiosynthesis methods for many reasons: there is less need for chemical reagents and precursors, leading to prevention of waste. Only a small amount of PET tracers is necessary (a single nanogram is sufficient for a full human body scan). There is a lower probability of exposure to radioactive materials when performing reactions. A large space, in relative terms, will not be necessary for performing radiosynthesis. The time it takes to perform radiosyntehsis can be shortened from a quicker and more efficient reaction, resulting from precise control of reaction variables including heating, transporting, and mixing of reagents.

There has been progress in the development of miniaturized devices and microscale reactions have been performed in such devices. However, there have yet to be extensive studies done on [^{18}F]FLT radiosynthesis [14]. Here, we studied several factors that affect the synthesis of [^{18}F]FLT in order to discover better reaction conditions for performing micro-scale reactions on miniature devices. This was done to produce a larger radiochemical yield of [^{18}F]FLT.

2. Results and discussion

2.1. Microscale radiosynthesis of [^{18}F]FLT

[^{18}F]FLT was prepared via fluorination of 3-*N*-Boc-5′-*O*-dimethoxytrityl-3′-*O*-nosyl-thymidine (DMTr-Boc-nosylate FLT precursor), followed by deprotection of the *tert*-butyloxycarbonyl (BOC) and the dimethoxytrityl (DMTr) groups as shown in Fig. 1. In this study, ^{18}F-substitution reaction was carried out at 100 °C with tetrabutylammonium bicarbonate ($TBAHCO_3$). Hydrolysis was performed at 95 °C with a 1.2 M HCl solution. To determine the overall efficiencies and yields at each reaction step, we measured the conversion yield and radioactivity caused by the reaction after each synthesizing step. Similar to the previous study, these reactions were performed on a Teflon-coated glass chip (see Fig. S1 in the supplementary data), which has the same geometry as EWOD devices [14].

The use of polar solvents containing the alcohol functional group in [^{18}F]fluorination has been reported to achieve high yields according to Kim and coworkers [15]. They found that higher [^{18}F]fluorination yields resulted from *tert*-alcohols than primary and secondary alcohols because of alcohol's catalytic role in the nucleophilic fluorination substitution reaction. In contrast to our method, their study was done by performing macro-scale [^{18}F]fluorination reactions at a high temperature (120 °C) and a relatively long reaction time of 10 min in synthesizing [^{18}F]FLT. In our study, we decided to use less harsh reaction conditions including a lower temperature of 100 °C and shorter reaction times. These values were used with variations in the types of bulky alcohols to optimize nucleophilic substitution reactions. Shorter reaction times resulted in increased radiochemical yield. As shown by Table 1, a variety of tertiary alcohols were tested. With these parameters, [^{18}F]fluorination yield in 2,3-dimethyl-3-pentanol and 3-methyl-3-pentanol were 92.7% and 92.1%, respectively, within 2.5 min. During our study, we found that one of the bulkier alcohols, 2-phenyl-2-butanol, effectively lowered the [^{18}F]fluorination yield, in comparison to other tertiary alcohols. This was an interesting result because we anticipated that [^{18}F]fluorination yield would increase from the higher steric hindrance. The opposite effect was attributed to the fact that the phenyl ring caused a highly rigid, steric hindrance effect that prevented direct nucleophilic substitution of the fluoride ion. Therefore, our results suggest that only flexible, aliphatic tertiary alcohols, and not rigid ones, can enhance the yield of [^{18}F]fluorination yield.

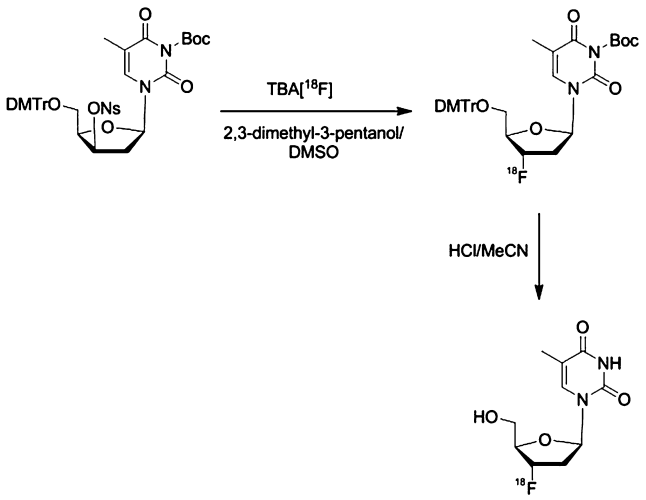

Fig. 1. Scheme of radiosynthesis of [^{18}F]FLT.

We examined the co-solvent effect of 2,3-dimethyl-3-pentanol on fluorination. Due to its low boiling point of 82 °C, MeCN completely evaporated when used as a co-solvent. Therefore, DMSO was chosen because of its higher boiling (189 °C) temperature than the mixture of 2,3-dimethyl-3-pentanol and MeCN. Since solubility is a crucial factor in chemical reactions, we verified the solubility of the DMTr-Boc-nosyl FLT precursor in each of the 2,3-dimethyl-3-pentanol/DMSO mixture. DMTr-Boc-nosyl FLT precursor was completely dissolved in 25–78% of 2,3-dimethyl-3-pentanol of the solvent mixture. Increasing the amount of 2,3-dimethyl-3-pentanol to 78% did not affect the solubility of the DMTr-Boc-nosyl FLT precursor. Also, it was noted

Table 1
Protic alcohol solvent effect on [^{18}F]fluorination yield.[a]

Solvent	[^{18}F]fluorination efficiency (%) (n = 3)			
	1.5 min	2.5 min	3.5 min	4.5 min
2,3-Dimethyl-3-pentanol	75.6 ± 2.6	92.7 ± 1.5	94.2 ± 1.3	94.9 ± 1.6
3-Methyl-3-pentanol	72.4 ± 2.3	92.1 ± 1.5	93.3 ± 2.3	94.2 ± 1.7
2-Methyl-2-pentanol	65.9 ± 4.2	91.5 ± 2.1	92.5 ± 2.7	93.1 ± 1.8
2-Phenyl-2-butanol	21.4 ± 3.2	39.4 ± 4.3	62.6 ± 2.6	76.7 ± 2.7

[a] Reaction condition: 54 mM of 3-*N*-Boc-5′-*O*-dimethoxytrityl-3′-*O*-nosyl-thymidine, 2:1.5 (mol/mol) DMTr-Boc-nosylate FLT precursor/base ($TBAHCO_3$), 100 °C.