3D プリンターとナノテクノロジーを駆使する環境調和型の新規酵素反応システムによる有用物質の合成

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Biocatalysis has been a promising and green approach for various industries. However, the limitations of free enzymes are their instability and unable to be recycled. Therefore, enzyme immobilization has been addressed to solve the problems. Nowadays, the advent of nanotechnology and material science have availed in the success of enzyme immobilization. In the meantime, 3D-printer technologies have significantly been developed due to their advantages, such as being versatile, convenient, time-saving, and inexpensive. Therefore, this research utilized nanotechnology and 3D-printer technology to develop new approaches for enzyme immobilization and beneficial compounds production (**Fig. 1**). Several novel enzymes were immobilized in/on novel support materials, for examples, organic-inorganic nanocrystal, graphene-based nanomaterials, and the surface of 3D-printed reactors. The immobilized enzymes exhibited superior properties to that of free enzymes. In addition, we successfully fabricated 3D-printed bioreactors and established a continuous flow process to produce an enantiopure alcohol. Eventually, we intend to produce beneficial compounds on a large scale from our developed continuous-flow biocatalytic system to promote green industries.

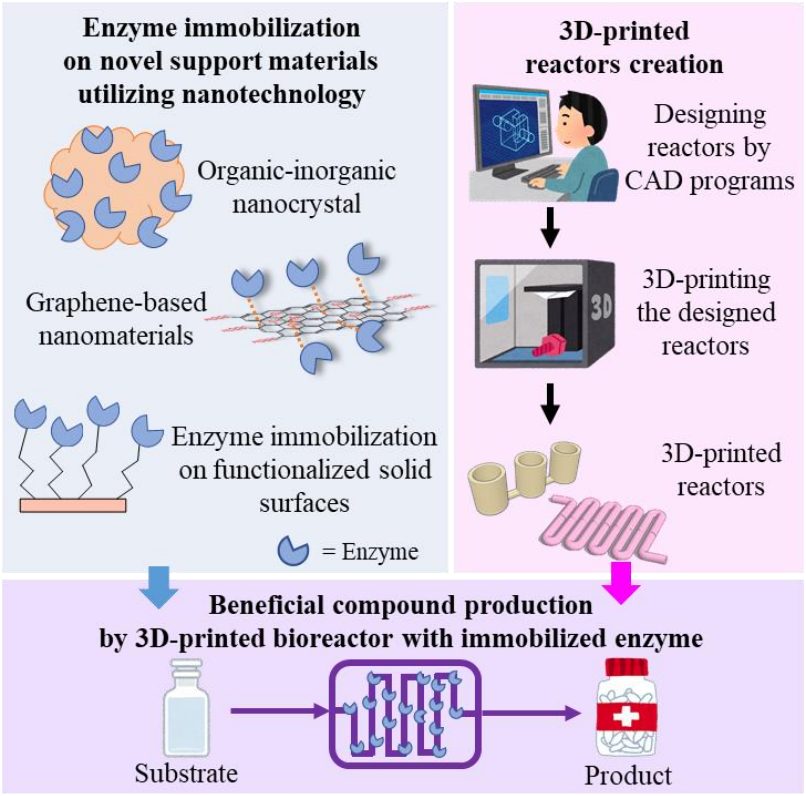

Fig. 1 Applications of nanotechnology and 3D-printer in enzyme immobilization for beneficial compound production

1. Organic-inorganic nanocrystal formation

This immobilization strategy is simple and effective. We successfully immobilized various novel enzymes such

as *Geotrichum candidum* acetophenone reductase (*Gc*APRD), *Geotrichum candidum* aldehyde dehydrogenase (*Gc*ALDH), and Baeyer–Villiger monooxygenase (*F*BVMO), by this method. We found that all of the enzyme nanocrystals exhibited superior properties to the free enzyme, such as better stability and recyclability. In addition, we synthesized beneficial compounds by *Gc*APRD nanocrystal and achieved a high reaction yield with excellent enantioselectivity (**Fig. 2**).

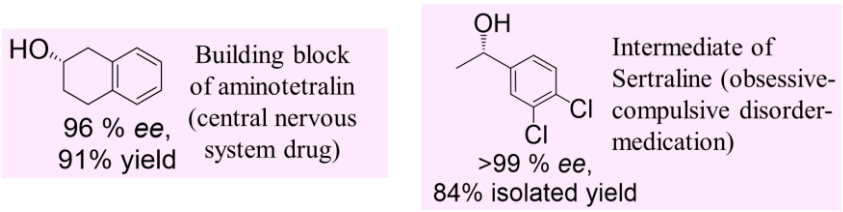

Fig. 2 Beneficial compounds produced by *Gc*APRD nanocrystal

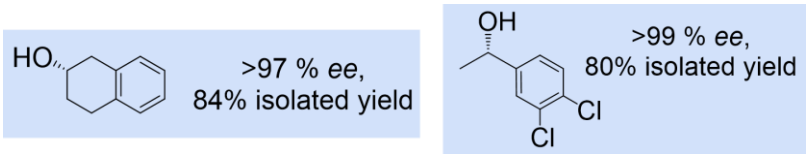

Fig. 3 Beneficial compounds produced by rGO-*Gc*APRD

2. Graphene-based nanomaterials

Graphene oxide (GO) and reduced graphene oxide (rGO) have been attractive carriers for enzyme immobilization because of their unique characteristics, for examples, strong mechanical strength and large specific area. Thus, we immobilized *Gc*APRD on GO and rGO *via* physical adsorption. It appeared that rGO loaded *Gc*APRD with a high immobilization yield (maximum ~3 mg protein/mg rGO) and retained high enzyme catalytic activity after immobilization (104%). This approach solved the trade-off relationship problems between immobilization yield and the retained enzyme activity found in general immobilization methods. **Fig. 3** shows examples of beneficial compounds synthesized by rGO-*Gc*APRD.

3. 3D-printed bioreactor of *Gc*APRD

We designed bioreactors by computer-aided design (CAD) programs and fabricated them with a 3D-printer using polypropylene as a material. Then, the surfaces of the 3D-printed reactors were functionalized by mussel-inspired polydopamine, glutaraldehyde, and polyethylenimine for *Gc*APRD immobilization. We found that the immobilized *Gc*APRD performed sufficient activity and excellent enantioselectivity after immobilization. Additionally, we successfully established continuous flow processes utilizing 3D-printed microfluidic bioreactors (**Fig. 4**) with immobilized *Gc*APRD to produce an enantiopure alcohol, and it could operate up to 117-144 h.

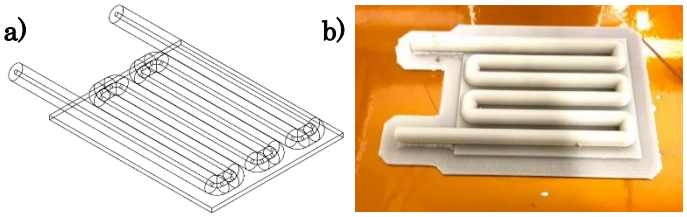

Fig. 4 a) CAD of the microfluidic bioreactor, and **b)** 3D-printed microfluidic bioreactor

要約：酵素は、様々な分野において、環境にやさしい触媒として有用である。しかし、遊離の酵素は不安定な場合もあり、また、再利用できないなどの問題がある。これらの問題を解決するためには、酵素を固定化する必要がある。本研究では、3D プリンターを利用し、また、ナノテクノロジーを駆使することにより、酵素の固定化に成功した。酵素としては、acetophenone reductase (*Gc*APRD)、aldehyde dehydrogenase (*Gc*ALDH)、および、Baeyer-Villiger monooxygenase (*F*BVMO)を用いた。固定化法としては、有機―無機ハイブリッドナノクリスタルを作成する方法、酸化グラフェン上に固定化する方法、および、3D プリンターで作成したリアクター表面に表面処理を行なった後に酵素を固定化する方法に成功した。さらにフロー系の反応にも成功した。