Functional Design of Bioadaptive Nano-Polysaccharide Architectures Constructed by Pickering Emulsion Templating

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Title　：Functional Design of Bioadaptive Nano-Polysaccharide Architectures Constructed by Pickering Emulsion Templating
（ピッカリングエマルション鋳型法による生体適応性ナノ多糖構造体の機能設計）

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Thesis Summary

Naturally occurring polysaccharides with adaptive and complex intrinsic bioactivities play an essential role as novel biomaterials in various biomedical fields, including tissue engineering, drug delivery systems and vaccine adjuvants. In particular, polysaccharide nanofibers with excellent flexibility and deformability, high aspect ratios, and tunable surface chemistry have attracted extensive attention in the construction of biomedical nano- and micromaterials. This dissertation focused on the functional design of polysaccharide nanofibers, such as cellulose, chitin and chitosan, using Pickering emulsion (PE) technology to fabricate bioadaptive nano-polysaccharide architectures including 3D cell culture scaffolds for liver cells and PE-based microparticles that act directly on cellular events.

First, a facile and novel strategy was devised to construct 3D porous scaffolds for cell culture by a PE templating and lyophilization method using TEMPO-oxidized cellulose nanofiber (TOCNF) and chitosan nanofiber (CsNF). The two-step emulsification process that an oil-in-water PE was firstly stabilized with longer CsNF, followed with shorter TOCNF greatly contributed to forming highly stable PE, which possessed a denser interfacial layer of oil droplets and strengthened network structures around the droplets. Porous scaffolds were successfully fabricated *via* lyophilization, and applied for culturing mouse fibroblast (NIH/3T3) and human liver carcinoma (HepG2) cells. Both of cells grew not only on the surfaces but also inside of the scaffolds, due to the high porosity structures and the functional glyco-endowed biointerfaces. Especially, HepG2 spheroids were formed inside and exhibited 10-fold higher enzymatic responses for detoxification than that cultured on conventional 2D tissue culture polystyrene substrate.

Next, the characteristic cytotoxicity and inflammatory responses of TOCNF-stabilized PE (TPE) and chitin nanofiber-stabilized PE (CPE) were discovered for NIH/3T3, HepG2 and mouse Kupffer (KUP5) cells. As compared with conventional β-tricalcium phosphate nanoparticle-stabilized or surfactants-stabilized emulsion microparticles, polysaccharide nanofiber-stabilized PE (TPE and CPE) induced higher lactate dehydrogenase (LDH) release in all the cells, following with a dose-dependent behavior, both of which induced the highest LDH release in HepG2 cells. Furthermore, characteristic pyroptotic cell death behavior, which is accompanied by cell swelling, membrane blebbing, caspase-1 activation as well as interleukin-1β (IL-1β) production, evidently occurred in TPE- or CPE-treated KUP5 cells. These PEs demonstrated biological activity as a mediator of the inflammation response,

which may provide new insight into regulating inflammation-related diseases for designing potent anticancer drugs and vaccine adjuvants.

To further elucidate the possible mechanisms mediated by nano-polysaccharides in PE systems, four types of nanofibers with different surface chemistry, such as inherent cellulose nanofiber (CNF), phosphorylated cellulose nanofiber (PCNF), sulfated cellulose nanofiber (SCNF) and TOCNF, were compared to prepare PE microdroplets, coded as CPE, PPE, SPE and TPE, respectively. Due to each morphological difference, CPE exhibited the largest droplet size, while PPE and SPE possessed the highest physical stability with smaller droplet sizes. The effect of PE microdroplet sizes on cytotoxicity of NIH/3T3 cells was investigated in detail by tuning oil phase ratios from 5 to 30 v/v%. Reactive oxygen species induction and cathepsin B (lysosomal cysteine protease) release in SPE-treated KUP5 cells were confirmed. High loading efficiency of ovalbumin as a model of antigen was achieved greater than 85%, owing to the high stability and large specific surface area of SPE. As-designed polysaccharide PE microparticles with high loading efficiency of antigen are expected to demonstrate excellent deformability and pliability to be efficiently taken up by macrophages, indicating a promising application for advanced vaccine adjuvants preparation.

To summarize, bio-based polysaccharide nanofibers derived from forest and marine resources were successfully employed as solid nano-stabilizers to construct highly stable Pickering emulsion with tunable morphology and bioadaptability. Nano-polysaccharide architectures as a template for 3D cell culture or vaccine adjuvants have greatly extended their utilization in biomedical applications, which go far beyond their conventional usage as low-value bulk materials. Natural, renewable and structural polysaccharide nanofibers instead of petroleum-based polymer materials can offer a promising future as functional, eco-friendly, biodegradable and sustainable biomaterials, which will open up a new avenue for realizing eco-society toward achieving the Sustainable Development Goals.