学 位 論 文 審 査 の 要 旨
Doctoral Dissertation Evaluation Review

博士の専攻分野の名称　博士（生命科学）　　氏　名

Degree requested: Doctor of （Life Science）　　Name: Md. Tariful Islam Mredha

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学 位 論 文 題 名
Title of Doctoral Dissertation

Designing biopolymer-based hydrogels with biomimetic superstructures
（バイオミメティック超構造を有する生体高分子ゲルの創製）

博士学位論文審査等の結果について（報告）
Results of Evaluation of the Doctoral Dissertation (Report)

Nature is an advanced school to learn biomaterial designing, where various biopolymers are found in different organized forms to regulate cellular activity and various specific bio-functions. Hydrogels, a class of soft and wet materials, are considered to be the most promising smart biomaterials due to their similarity to soft biotissues. The scope of hydrogels is often prevented due to their amorphous structure, resulting in the absence of many functions. During tissue formation, different rigid biopolymers (such as, collagen, cellulose, alginate, chitosan *etc.*) secret from the cell and organize hierarchically from their molecular scale to their macroscopic scale by sensing some mechanical signals from the cell. Based on this bio-motivated principle, this dissertation develops different kinds of *in vitro* systems, where mechanical signal could be generated with tunable strength by controlling the bottom-up growth kinetics of polymers and in this way, successfully designed various kinds of biomimetic superstructures in hydrogel network using those biopolymers.

Collagen is the main component of connective tissues and is the main abundant protein in the mammals. Marine collagen has been attracting attention as a medical material in recent times due to the low risk of pathogen infection compared to animal collagen. The author has found that, type I collagen extracted from the swim bladder of Bester sturgeon fish (a hybrid sturgeon of *Huso huso* x *Acipenser ruthenus*) has excellent characteristics such as high denaturation temperature, high solubility, low viscosity and an extremely fast rate to form large bundle of fibers under certain conditions. Those specific characteristics of swim bladder collagen (SBC) have been utilized to develop different types of superstructures in hydrogel network. The author has created stable, disk shaped hydrogels with concentric orientation of collagen fibers, for the first time, by the controlled diffusion of neutral buffer through collagen solution at room temperature. However, traditionally used animal collagens, *e.g.* calf skin collagen (CSC) and porcine skin collagen (PSC), could not form any stable and oriented structure by this method. The author explored the mechanism that, the internal stress generated from the bottom-up growth process in the *reaction-diffusion* (RD) system of rigid polymer was responsible for superstructure formation. Later on, the author designed a bio-inspired novel method for generating strong mechanical signal with tunable strength to create long-range hierarchical structure in hydrogel network. In this process, mechanical signals could be transferred through *pulsatile diffusion* process in a RD system to create periodic stripe pattern in hydrogel network. This novel method has been applied

universally for other biopolymeric systems, such as chitosan, carboxymethyl cellulose and alginate; however, the sensing capability of diffusion pulse depends on the main chain rigidity of polymer, which ultimately affects the superstructure patterns. By tuning the diffusion kinetics of ions through SBC solution, the author has also successfully developed tunable superstructures, such as, concentric and radial orientation pattern and their combination at different percentages in the disc-shaped SBC hydrogels. In another study, the author has created complex twisting superstructures in cylindrical shaped SBC hydrogel by *free injection* method. The twisted structure similar to the compact bone osteon has been generated by the combination of two mutually orthogonal effects of injection shear and salt diffusion. Collagen hydrogels made from marine-based atelocollagen, having various kinds of biomimetic superstructures, self-standing capability and high thermal stability, will be suitable for cell culture and different soft tissue applications.

Load bearing tough connective tissues, such as ligament and tendon contain highly ordered hierarchically aligned fiber structures and high polymer density, and therefore can perform most of the load bearing activities in the human body. To create tendon like materials, the author proposed a facile and novel method. The strategy is simply the drying of physical hydrogel under confined condition; by which a spontaneous and strong internal stress is generated in the hydrogel to create tendon-like highly ordered hierarchy and simultaneously, high polymer density is achieved with drying process. Irreversible and permanent fiber formation occurred by the contraction of aligned polymer chains in hydrogels during drying process. By applying this strategy, the author succeeded to produce perfectly aligned fibrous structures with several orders of hierarchical sub-structures in alginate hydrogel. This hydrogel shows robust mechanical properties (elastic modulus > 400 MPa and fracture stress > 20 MPa) similar to the natural load-bearing tissues (ligament and tendon). The author is also succeeded to create highly aligned fiber in cellulose and collagen hydrogel by this method. This strategy could be generic for other biopolymeric hydrogels providing some critical conditions. This study would open a new avenue of materials science for designing biopolymer-based perfectly aligned fibrous hydrogel.

In brief, this dissertation has successfully designed several novel biomaterials with different kinds of biomimetic superstructures. The author has introduced a new marine collagen with excellent characteristics. The author has utilized those superior properties smartly for creating novel biomaterials with excellent functionalities. The author has developed a novel universal method for creating periodic stripe pattern with long-range hierarchical structure in hydrogel network. At the end, the author has introduced another novel method for creating perfectly aligned fibrous hydrogel with tendon-like periodical hierarchical structures and robust mechanical properties. This study integrates the cutting edge technologies of biomaterials, which will make a tremendous impact on medical science with the possibility of applying those biomimetic hydrogels for biomedical applications. The author contributed to the basic science, material science and biological science and also opens a vast area for designing future generation materials. Therefore, the author of this doctoral dissertation is qualified to be granted the Doctorate of Life Science from Hokkaido University.