

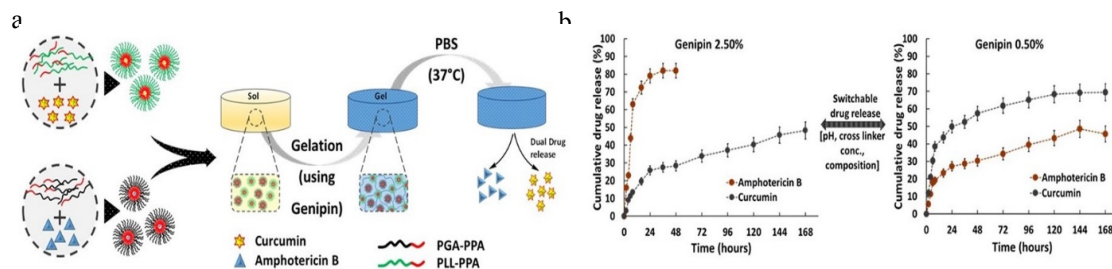
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論 文 題 目	Tunable polypeptide systems and their application in controlled delivery of drugs and cells (薬剤及び細胞の放出制御を目指したポリペプチド系の構築)		
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## 論文の内容の要旨

**Background:** In the current situation of drug delivery and therapeutics, combination drugs are progressively being developed to improve the curative effect and patient compliance. Several approaches have been employed to co-deliver drugs through a single system, such as multi-shell particles, hydrogels etc. But, to accelerate wound healing and decrease the side effects, a system which can release multiple drugs in suitable doses over a period of required time is the current need. Thus, it is highly desirable, to develop a drug delivery system that can control the release of multiple drugs with distinct release kinetics. For this, we designed a facile polypeptide based dual drug delivery system composed of micelle hydrogel composite.

**Results and Discussion:** Firstly, two differently charged amphiphilic polypeptides were prepared via ring opening polymerization of their specific amino acid NCAs (N-carboxy anhydrides). Thus formed polymers were cationic poly (L-lysine-*b*-L-phenylalanine) called PLL-PPA, and anionic poly (L-glutamic acid-*b*-L-phenylalanine) called PGA-PPA. These self-assembling polymers of PLL-PPA and PGA-PPA were loaded with Curcumin (Cur) and Amphotericin B (AmpB) to form stable micelles. The mixture of these drug loaded micelles was then further cross-linked using the free -NH<sub>2</sub> groups in PLL-PPA to successfully yield micelle hydrogel composite (Fig. 1a) with cross-linked PLL-PPA micelles and trapped PGA-PPA micelles. Thus, formed composites were studied for drug release properties.

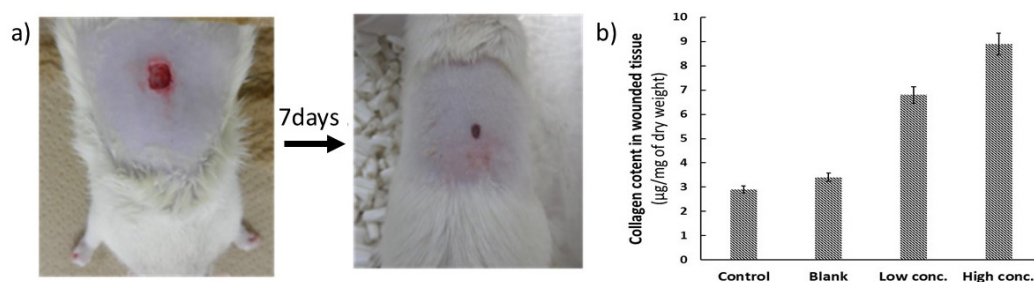


**Figure 1** a) Schematic representation of formation of drug loaded micelle-hydrogel composites, b) profile switching of the composites at different cross linker concentration.

The formed drug loaded PLL-PPA and PGA-PPA micelles were found to have spherical morphology with high drug loading efficiency (~80%) and were readily soluble in aqueous medium being stable for up to 14 days. The prepared composites were studied for different parameters: pH, cross-linker conc. and surface potential. Interestingly, the different environment and charges in the two different micelle components showed controllable yet opposite release kinetics of the drugs. As shown in Fig. 1b, when the micelle-hydrogel composite was subjected to varying degree of crosslinking, the drug release profile of both the drugs showed a switching among themselves. Cur loaded in PLL-PPA which showed lower drug release at low cross linker concentration switch its profile at higher cross linker concentration where as an opposite trend is seen for Amp B loaded in PGA-PPA.

In summary, a polypeptide based facile drug loaded micelle hydrogel composite was successfully formulated. The release results demonstrated that simple parameters such as pH, resultant zeta potential and crosslinking degree determined release profiles of drugs. And a unique switchable kinetics is observed in drug release profile of drugs.

To further evaluate drug release *in vivo*, rat models were used, excision wounds were created and hydrogel implant was made to cover the wounds and test the release of drug and wound healing. For the same, four different animal groups were created i.e. control (no gel), blank (gel without drug), low concentration (gel with curcumin 1mg/ml) and high concentration (gel with 2.5 mg/ml curcumin). Within 7 days wound size showed a significant decrease among the test animals, as well as increased collagen content was seen in the scared tissue compared to the control groups. And, in rat models tested with high concentration of drug in the gel showed a significant 1.5 times higher collagen content in comparison to control models corresponding to faster healing (figure 2).



**Figure 2.** In-vivo testing of the hydrogel composites (a) wound reduction in rat model on application of drug loaded hydrogel-micelle composite; (b) amount of collagen in the wounded tissue sample from the animals.

Microencapsulation within hydrogel microspheres holds much promise for drug and cell delivery applications. Synthetic hydrogels have many advantages over more commonly used natural materials such as alginate<sup>1</sup>, however their use has been limited due to a lack of appropriate methods for manufacturing these microspheres under conditions compatible with sensitive proteins or cells.

In the meanwhile emulsion techniques such as electrospray has risen as an answer to it. And encapsulation with this technique not only over comes the drawbacks of conventional encapsulation techniques but also provides additional benefits of size control and homogeneity. In this study investigated the use of synthetic polypeptide polymers to form cell encapsulating microspheres via submerged electrospray technique.

L929 murine fibroblasts were cultured in Eagles Minimum Essential Media (EMEM) supplemented with 10% foetal bovine serum and 100 mg/ml penicillin/streptomycin. When required for experiments, cells were trypsinized and re-suspended in PBS. The polymer solution of poly (L-lysine-co-L-glutamic acid) was dissolved in sterile PBS and the cell suspension was added to give a final cell concentration of  $1 \times 10^6$  cells/mL and 5 wt% of polymer. Sterile tetrakis (hydroxymethyl) phosphonium chloride (THPC) was added to a final concentration of 0.05wt% and the solution gently mixed to ensure even cell distribution. The whole solution was transferred to a syringe and electro sprayed.

Spheres produced were smaller for higher voltages and mean size could be tailored from 10 to 300µm. The microspheres exhibited a smooth, spherical morphology, did not aggregate. Also, the L929 fibroblasts were encapsulated within the polypeptide microspheres and showed viability >90% after 24 h and almost 80% viability after 3 days. Cell viability showed no visible influence by the high voltage proving it safe method of cell encapsulation.

Also, the polymer concentration was seen to influence the size of microsphere formation may be due to change in viscosity as the viscous force at meniscus drive the formation of microsphere by electrospray.

But still the size was under tailored limits. This process shows great promise for the production of synthetic hydrogel microspheres, and specifically supports encapsulation of cells. The polymeric system shows high cell viability and can be tuned for various sizes of microsphere.

**Keywords:** Synthetic polypeptide, drug delivery, controlled release, wound healing, cell encapsulation

## 論文審査の結果の要旨

本論文は、ポリペプチドにより形成されたハイドロゲルを用いることによって、薬物や細胞の新しい放出制御に関して詳細に検討したものである。

親水性モノマーと疎水性モノマーからなるブロック共重合体は、水中でミセルを形成することがわかっており、ミセルのコアに疎水性薬物を保持することが可能である。本論文では第 2 章において、2 種類の別々の薬物の徐放性を独立に制御できるハイドロゲルの分子設計を報告した。すなわち、リジンとフェニルアラニンからなるカチオン性両親媒性ブロック共重合体およびグルタミン酸とフェニルアラニンからなるアニオン性両親媒性ブロック共重合体を、 $\alpha$  アミノ酸 N-カルボキシ無水物(NCA)を用いた開環重合により合成し、二種類のミセルを作成した。カチオン性およびアニオン性の両親媒性ブロックポリペプチドに、抗菌剤であるアンホテリシン B と創傷治癒効果のあるクルクミンをそれぞれ別々に担持させ、カチオン性のブロックポリペプチドのアミノ基同士をゲニピンという架橋剤で化学架橋することでハイドロゲルを形成した。アニオン性のミセルは架橋されておらず、静電的相互作用でゲルの中に存在する。このゲルを異なる pH 環境に置くことでそれぞれのミセル内からの薬物の放出を独立に制御することが可能となった。低 pH 環境下ではアニオン性のミセルがプロトン化することで構造変化を起こし、崩壊することで内部の薬物を速やかに放出するのに対し、カチオン性のミセルは安定に存在するため、内部の薬物の放出はゆっくりと行われる。一方、塩基性条件下ではその逆の薬物放出プロファイルとなる。また、架橋剤濃度やミセルの混合比によっても内部の薬物の放出挙動が異なるなど、条件により自在に二種類の薬物の放出挙動を制御できることを示した。第 3 章では、その二剤放出制御ゲルの創傷治癒効果を動物実験で確かめた。ラットの背部に創傷モデルを作成し、薬物を封入したゲルを貼付し、8 日間の創傷治癒効果を、創傷部位の観察、真皮の再生、炎症の度合い、肉芽形成、血管新生、抗酸化活性、コラーゲン再生量等を指標として評価した。その結果、薬物封入ミセルゲルで有意に高い創傷治癒効果が得られた。これは、創傷部位の炎症による pH 低下に伴う早期の抗菌剤の放出に加え、持続的な創傷治癒剤の徐放によるものと結論づけられた。第 4 章では、両性ポリペプチドと細胞および架橋剤を混合した液をエレクトロスプレー法によりマイクロスフィア化し細胞のカプセル化を行った。その際のノズル径、電圧、架橋剤密度などによりカプセルの大きさや細胞への傷害性が異なるため、それらを最適化し細胞デリバリー用のカプセル化材料としての可能性を見いだした。

本論文は、両親媒性ポリペプチドの分子設計を基盤とした新たな薬物および細胞デリバリー材料としての可能性を詳細に検討し、有用性の高い材料応用への道筋を開いたという点で、学術的

に貢献するところが大きい。よって博士（マテリアルサイエンス）の学位論文として十分価値あるものと認めた。