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研究課題名（和文）A new glucose-responsive injectable hydrogel for mesenchymal stem cells transplantation to rat myocardial infarction heart

研究課題名（英文）A new glucose-responsive injectable hydrogel for mesenchymal stem cells transplantation to rat myocardial infarction heart

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交付決定額（研究期間全体）：（直接経費）　　2,300,000円

研究成果の概要（和文）：間葉系幹細胞を用いた心筋梗塞治療を目的として、ボロン酸ポリマーとポリビニルアルコール、ソルビトールからなるin situハイドロゲルシステムを開発した。この溶液は、細胞と混合して組織へ注入することでソルビトールの拡散によりMSCを保持しながら自発的にゲル化形成する。これらの材料は、いくつかの種類の細胞に対して細胞毒性がないことが判明しています。心筋梗塞モデルを用いて、この材料を注入することで心臓の有害なリモデリングを防ぎ、心機能が維持されることを実証した。

研究成果の学術的意義や社会的意義
The findings of this study suggest that sorbitol-responsive injectable hydrogel may be a novel therapeutic approach for myocardial infarction. Moreover, this study proposes a material concept that may surmount the low retention of cells and drugs to the target tissue.

研究成果の概要（英文）：A new sugar-responsive injectable hydrogel was developed for the treatment of myocardial infarction by combining it with or without mesenchymal stem cells (MSCs). We achieved the proof-of-concept in which the material solution included poly (3-acrylamidophenylboronic acid-co-acrylamide) (BAAm), poly(vinyl alcohol) (PVA), and sorbitol can become an in-situ gel after injection into the tissue via simple diffusion of the sorbitol to surrounding tissue. It has been found that those materials have no cytotoxicity with several cell types involving MSCs, smooth muscle cells, and fibroblast cells. Using a myocardial infarction model, we demonstrated that intramyocardial injection of this material prevents adverse cardiac remodeling, resulting in preserved cardiac function. The current data was published in a peer-reviewed journal and presented at six domestic and international scientific conferences.

研究分野：biomaterial

キーワード：injectable hydrogel　myocardial infarction　sorbitol response　adverse remodeling　MSCs　material distribution　material retention　cardiac function

１．研究開始当初の背景

(1) Myocardial infarction (MI), a leading cause of world mortality, leads to the death of cardiomyocytes. The loss of cardiomyocytes results in a sequence of adverse remodeling, including exacerbated left ventricular (LV) dilation, decreased thickness of the LV wall, and scar formation, eventually causing heart failure. Once advanced heart failure occurs, heart transplantation is the only option[1]. Currently, regenerative therapies using stem cells have been proposed to suppress adverse heart remodeling and promote de novo cardiac repair. However, it is challenging to deliver the cells to the beating heart with high efficiency and low invasiveness.

(2) Injectable hydrogel, made from highly hydrated polymers, can closely resemble the physical properties of many soft tissues and encapsulate living cells in its 2D or 3D structure. It has been thereby proposed as a novel strategy for the treatment of MI by combining with/without cells or drugs[2]. To date, many injectable hydrogels have been proposed involving natural hydrogels (e.g. chitosan, alginate, and fibrin) and synthetic hydrogels (e.g. PNIPAAm, poly(lactic-co-ethylene glycol)[2]. To overcome the excessive material viscosity before injection, the in-situ gelling materials, which can be injected as a liquid and become an in-situ gel after injection, have been recently attractive. However, the sol-gel transition of the reported materials is switched by pH, temperature changes, or ultraviolet light, which is difficult to drive in vivo and might impact the biological properties of encapsulated cells.

(3) Phenylboronic acid has been widely known as a crosslinker with the diol group of polyvinyl alcohol (PVA) to form a hydrogel network and with the diol group of sugars involving glucose and sorbitol[3]. It has been shown that boronic ester-based hydrogel is a sugar-responsive biomaterial that is used in drug and cell delivery systems. In such systems, the encapsulated drug is released as the hydrogel disintegrates by adding sugar[4]. The presence of sugar has been shown to block the crosslink of phenylboronic acid-grafted polymer with PVA to give a sol phase because it has a higher affinity with boronic acid.

(4) Recently, we successfully synthesized poly (3-acrylamidophenylboronic acid-co-acrylamide) (BAAm) and prepared a solution including BAAm, PVA, and sorbitol (BAAm/PVA/S or S-BAVA). Since the crosslinking between the boronic acid and the diol is unstable, we hypothesized that this material solution could be gelled after injection into the tissue by simply diffusing the sorbitol from the sol to the surrounding tissue

(5) Mesenchymal stem cells (MSCs), a multipotent cell type, have been commonly used for cell therapy in MI in both basic research and clinical trials. MSCs have a powerful secretion of biological factors and immunomodulatory ability, although their differentiation to cardiomyocytes is poor.

２．研究の目的

This study aimed to clarify the sol-gel transition of the S-BAVA solution upon contact with the tissue to investigate the role of this hydrogel in the delivery of drug and cells as well as its therapeutic effects in the prevention of adverse LV remodeling and the maintenance of cardiac function after MI.

３．研究の方法

(1) Synthesis and characterization of BAAm and rhodamine-conjugated BAAm polymer

The 3-acrylamidophenylboronic acid (AAPBA), acrylamide (AAm), and azodiisobutyronitrile were dissolved in an ethanolic solution of 40 v/v% and reacted at 60℃ for 18 hours. The resultant solution was dialyzed for 3 days and freeze-dried. To conjugate rhodamine to BAAm (rho-conjugated BAAm, rho-BAAm), methacryloxethyl thiocarbamoyl rhodamine B monomer was added to a mixture of AAPBA and AAm monomer, and the polymerization was done in the same condition with BAAm. The characterization of BAAm and rho-BAAm polymer was examined by ^{1}H NMR and GPC.

(2) Preparation and mechanical properties of S-BAVA

The S-BAVA solution comprised 25 mg/mL of BAAm, 10 mg/mL of PVA, and 10 mg/mL of sorbitol. The viscosity and modulus of S-BAVA at different concentrations of sorbitol from 0 to 10 mg/m were measured by rheometer.

(3) The tissue adhesive ability of BAAm/PVA (BAVA) hydrogel
Using a decellularized vascular graft, we tested the tissue adhesive ability of the BAVA hydrogel, including 10 mg/mL of PVA and 25 or 50 mg/mL of BAAm. The BAVA hydrogel was loaded on the adventitial surface of two grafts. Then, the lap-shear test was done to measure the mechanical tissue adhesive curve of the BAVA hydrogel.

(4) The changes in sorbitol concentration of S-BAVA solution in the porcine tissue in vitro
The S-BAVA solution having 10 mg/mL of sorbitol was added to the holes in the porcine heart tissue. The solution was collected at 5, 15, 30, 60, 90, and 120 min after incubation in the tissue. The sorbitol concentration of the collected samples was measured by an EnzyChromTM sorbitol assay kit.

(5) The cell viability of MSCs when it was co-cultured with S-BAVA
The MSCs at passengers 3 or 4 were seeded in one layer in the culture dish. After overnight culture, the S-BAVA with different concentrations of sorbitol was added to the culturing cells (100 uL of material per 100 ul of medium). The cell viability was examined by a cell-counting kit 8 at 24h after co-culturing with the material. The cell viability in the medium, including 100 ul of PBS, was set as a control.

(6) Tracking the cell viability after intramyocardial injection of MSCs-loaded S-BAVA solution into the beating heart
The MSCs were labeled with 8-arm PEG-FITC-Gd^{+}, a contrast reagent for 7T-MRI that was successfully developed in our laboratory. The labeled MSCs suspension solution was mixed with the sorbitol solution to gain 10^6 cells per 25 uL of sorbitol solution (40 mg/mL). Then the cell-sorbitol mixture was mixed with 25 ul of BAAm solution (100 mg/mL) and then mixed with 50 ul of PVA solution (20 mg/mL). The final solution included 25 mg/mL of BAAm, 10 mg/mL of PVA, 5 mg/mL of sorbitol, and 10^6 labeled MSCs (MSC-loaded S-BAVA solution). The MSC-loaded S-BAVA solution was intramyocardially injected into the rat's beating heart. At 1h after injection, the cell viability in vivo was tracked by 7T-MRI or the heart was collected for cell observation with fluorescent microscopy.

(7) The distribution and retention of rho-labeled S-BAVA hydrogel in the infarction tissue
A rat subacute MI model was prepared by surgically ligating left anterior descending (LAD) artery. The rho-labeled S-BAVA solution was prepared from 25 mg/mL of rho-conjugated BAAm, 10 mg/ml of PVA, and 10 mg/mL of sorbitol. The 200 ul of rho-labeled S-BAVA solution with or without PVA was intramyocardially injected in the infarction area of LV wall. The presence of the injected materials was observed under fluorescent microscopy.

(8) The therapeutic effect of S-BAVA hydrogel injection in a rat MI model.
Using three sets of MI models,200 ul of S-BAVA or calcium-crosslinked alginate (ALG) or saline solution was injected into the LV infarction wall (four to six rats per group). The cardiac function was examined by echocardiography every one week for up to three weeks. The cardiac morphology was assessed at three weeks after treatment by Hematoxylin and Eosin (H&E) and Trichrome staining. The saline group was set as a negative control.

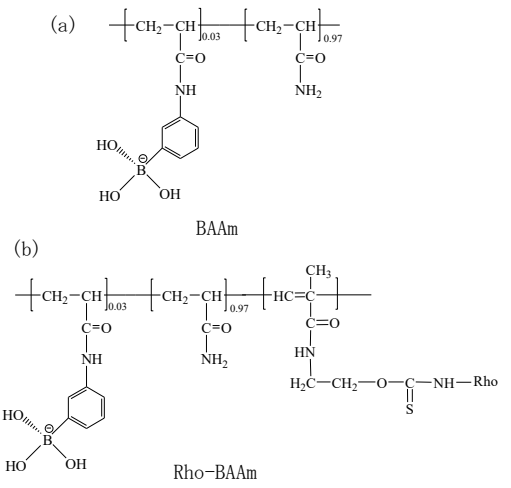

Figure 1. The chemical structure (a) of BAAm polymer, (b) of rho-conjugated BAAm polymer.

４．研究成果

(1) Synthesis and characterization of BAAm and rho-conjugated BAAm polymer

The chemical structures of BAAm and rho-conjugated BAAm polymer are shown in Figure 1. The molecular weight (Mw) and polydispersity index (Mw/Mn) of the BAAm polymer determined by GPC were 107kDa and 3.7, respectively. The Mw and Mw/Mn of the rho-BAAm polymer were 71kDa and 4.1, respectively. The 1H NMR spectrum showed that The molar ratio of AAPBA to AAm in the BAAm was as same as those in the rho-BAAm.

(2) Mechanical properties of S-BAVA with different concentrations of sorbitol

The S-BAVA with 10 or 5 mg/mL of sorbitol was in the sol state. The sol state of S-BAVA was transited to the gel state when the sorbitol concentration was reduced to less than 1.25 mg/mL (Figure 2a). The rheological tests showed that the loss and storage modulus of the S-BAVA, which was measured at a fixed condition of strain 3% and frequency 6Hz, was increased as the sorbitol concentration in the S-BAVA was decreased. The maximum loss and storage modulus of the S-BAVA hydrogel reached approximately 50 and 100 Pa, respectively. In addition, the viscosity of the S-BAVA also gradually increased, depending on the sorbitol concentration in the S-BAVA (Figure 2b). In summary, the data indicated that the gelation of the S-BAVA solution was switched by a reduction of sorbitol concentration.

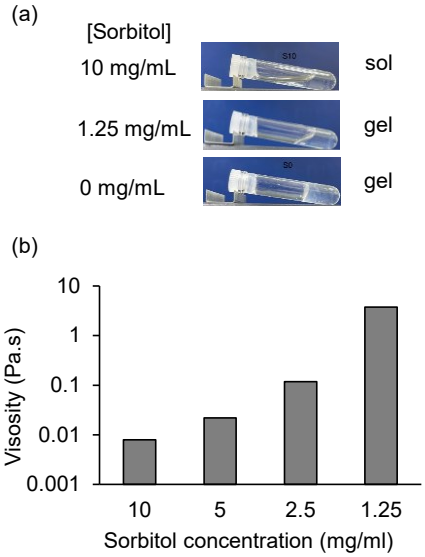

Figure 2. (a) The photograph of S-BAVA with different concentrations of sorbitol. (b) The viscosity of S-BAVA in response to the changes in sorbitol concentration at shear rate 1 s^{-1}

(3) The tissue adhesive ability of the BAVA hydrogel

The lap-shear test showed that the BAVA hydrogels could adhere to the adventitial surface of the decellularized vascular graft. When comparing with the BAVA hydrogels including 50 mg/mL of BAAm, the BAVA hydrogel including 25 mg/mL of BAAm revealed a higher tissue adhesive ability. Since the tissue adhesive ability of the material has reportedly increased the localization of the loaded drug in the target tissue, we suggested that the BAVA25 hydrogel might be a good candidate for cell delivery to the target tissue.

(4) The sorbitol concentration in the S-BAVA solution after adding it to the porcine heart tissue

The sorbitol concentration in the S-BAVA solution was spontaneously reduced as time contact with the tissue. The reduced sorbitol reached 38 % at 15 min and 60 % at 120 min. Parallelly, the sol-gel transition of the S-BAVA solution in the tissue was also observed. The sol transited to high viscous sol at 60 min and formed gel at 150 min. Taken together, those data suggested that the gelation of the S-BAVA solution automatically occurred via simply diffusing the sorbitol molecule from the solution to the body.

(5) The MSCs viability when co-culturing with the S-BAVA hydrogel

The cell viability after incubation with the S-BAVA hydrogel is shown in Figure 3.The ratio of MSCs viability to the control was over 100% when the cells were co-culturing with the S-BAVA hydrogels, including different concentrations of sorbitol ranging from 0 to 10 mg/mL for 24h. Those data indicated that the S-BAVA

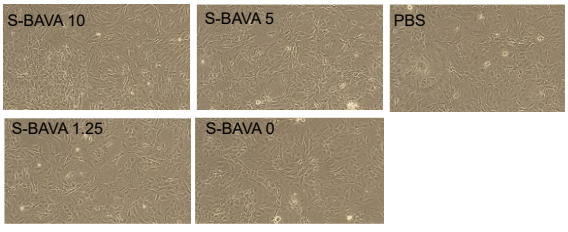

Figure 3. The optical images of the MSCs co-culturing with the S-BAVA hydrogels including 10, 5, 1.25, 0 mg/mL of sorbitol and with PBS.

hydrogel had no cytotoxicity on the MSC.

(6) Tracking the MSCs viability in vivo

In the preliminary test, we failed to observe cell viability in vivo by the 7T-MRI technique. A reason for this failure might be that the contrast reagent's dose used was not effective in the T2 phase of 7T-MRI, thereby an optimization of the contrast reagent's dose is suggested in future research. In another test, the fluorescent images showed the presence of the cells in the heart tissue at 1h after injection with labeled cell-loaded S-BAVA hydrogel. However, the cell was not observed in the case administrated with only labeled cells. Those preliminary data suggested that the S-BAVA hydrogel had the potential for improvement of cell viability in vivo.

(7) The distribution and retention of rho-labeled S-BAVA hydrogel in the infarction tissue

The S-BAVA without PVA can fully cover the infarction area 1 h after injection, but it sharply disappeared at 1 week. In contrast, the S-BAVA solution was not only widely distributed in the infarction area but also remained for up to 3 weeks. Those data suggested that the gelation of S-BAVA might occur after injection, elongating its retention in heart tissue.

(8) The therapeutic effect of S-BAVA in a rat MI model

A gradual reduction of the LV function indicated by a declining fraction shortening (FS) was observed in the saline-injected MI rats. Even with the ALG injection, the reduction of FS still occurred, although it was less severe than in the cases with saline injection. However, the FS in the S-BAVA group was not reduced for three weeks (Figure 4). In addition, the increase of systolic LVID caused by MI was suppressed by the S-BAVA injection, whereas the saline or ALG injection did not. The histological assessment showed that the infarction area in the S-BAVA group was lower than that in the ALG and saline groups after 3 weeks of administration. Intramyocardial injection of the S-BAVA solution effectively suppressed adverse LV remodeling, resulting in maintaining cardiac function for up to 3 weeks.

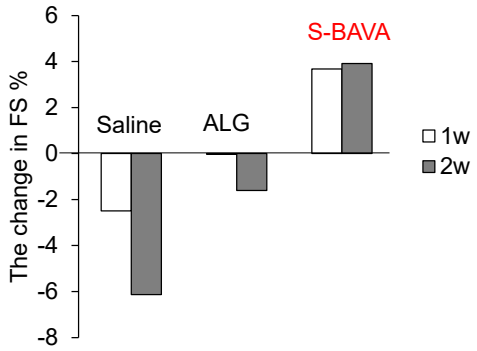

Figure 4. The change in FS index 1- and 2-week post-treatment with S-BAVA, ALG, and saline injection (compared to pre-treatment)

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５．主な発表論文等

〔雑誌論文〕　計2件（うち査読付論文　1件／うち国際共著　1件／うちオープンアクセス　0件）

１．著者名 Hue Thi Le, Atsushi Mahara, Takeshi Nagasaki, and Tetsuji Yamaoka	４．巻 147
２．論文標題 Prevention of anastomotic stenosis for decellularized vascular grafts using rapamycin-loaded boronic acid-based hydrogels mimicking the perivascular tissue function	５．発行年 2023年
３．雑誌名 Biomaterials Advance	６．最初と最後の頁 213324
掲載論文のDOI（デジタルオブジェクト識別子） 10.1016/j.bioadv.2023.213324	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Hue Thi Le, Atsushi Mahara, Kyoko Fukazawa, and Tetsuji Yamaoka	４．巻 41-2
２．論文標題 Challenges of new therapeutic applications for cardiovascular diseases by hydrogel injection	５．発行年 2023年
３．雑誌名 Journal of Japanese Society for Biomaterials	６．最初と最後の頁 114-117
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 無
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

〔学会発表〕　計6件（うち招待講演　0件／うち国際学会　2件）

１．発表者名 Hue Thi Le
２．発表標題 The effect of sorbitol-responsive injectable hydrogel on cardiac function and morphology of MI rat
３．学会等名 the 2022 JSB/SFB Joint Symposium（国際学会）
４．発表年 2022年

１．発表者名 Hue Thi Le
２．発表標題 Development of sorbitol-responsive injectable hydrogel for treating myocardial infarction
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The 51st Research Group on Biomedical Polymers

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Hue Thi Le

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３．学会等名
第44回日本バイオマテリアル学会

４．発表年
2022年

１．発表者名
Hue Thi Le

２．発表標題
Sorbitol-releasing in situ gelling materials for treatment of myocardial infarction

３．学会等名
第72回高分子学会年次大会

４．発表年
2023年

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関