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Title	Establishment of a Starch-Based Support Material with Interfacial Crosslinker Delivery for 3D Bioprinting of Functionalized Gelatin Bioinks
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Form 3

Abstract of Thesis

Name （Pekik Wiji Prasetyaningrum）	
Title	Establishment of a Starch-Based Support Material with Interfacial Crosslinker Delivery for 3D Bioprinting of Functionalized Gelatin Bioinks (ゼラチン誘導体インクを用いた3Dバイオプリンティングのための界面からの架橋剤送達を可能にするデンプン系犠牲支持材料の開発)

Abstract of Thesis

Functionalized gelatin derivatives are attractive materials for use as bioinks in three-dimensional (3D) bioprinting due to their ability to maintain the instructive properties of gelatin while enabling stable covalent crosslinking. Nonetheless, gelatin derivatives often lack the rheological properties required for bioink printability, posing challenges for cell-laden construct fabrication via extrusion-based 3D bioprinting. Hence, this dissertation addresses that challenge by establishing a starch-based sacrificial support material that simultaneously provides physical support, delivers crosslinking agents across the support–bioink interface, and can be removed under mild enzymatic conditions using α-amylase.

Prior to developing the printing strategy, in Chapter II, the functionalization-associated cellular responses of the gelatin derivatives were examined independently of crosslinking. Fibroblasts (BALB/3T3), cervical cancer cells (HeLa), mesenchymal stem cells (UE7T-13), and neuronal cells (PC-12) were exposed to soluble gelatin methacrylate (GelMA), gelatin phenol (GelPH), and gelatin norbornene (GelNB) at matched polymer concentrations and comparable degrees of functionalization (~50%). Using mitochondrial activity, proliferation, morphology, phenotype-related gene expression, and osteogenic differentiation assays, this study suggested that even in the absence of crosslinking, different gelatin functionalizations may modulate distinct, cell-dependent responses. Under the tested conditions, GelMA induced the most noticeable cellular changes, GelPH showed intermediate responses, and GelNB exhibited responses most similar to the control. These findings provide a basis for selecting derivatives for subsequent bioprinting.

In Chapter III, an extrusion-based 3D bioprinting strategy was developed by alternately extruding a starch support material and GelPH bioink. The wheat starch slurry conditions were refined to obtain a support material with suitable rheological properties and the ability to function as a crosslinker reservoir. By incorporating hydrogen peroxide (H_2O_2) into the starch slurry and horseradish peroxidase (HRP) into the bioink, GelPH crosslinking was achieved through interfacial H_2O_2 diffusion. The approach was applied to print UE7T-13 mesenchymal stem cells, and the printed cells retained viability and preserved their stemness-related characteristics and osteogenic differentiation capacity within the fabricated constructs.

In Chapter IV, the developed 3D bioprinting strategy was extended to GelNB bioinks by loading the starch support material with the thiol crosslinker dithiothreitol (DTT). Delivering DTT through the support material enabled visible-light-mediated thiol–ene crosslinking of GelNB constructs while minimizing direct cell exposure to the cytotoxic crosslinker. In this study, the indirect DTT supply method was compared with a direct DTT premixing method, in which DTT was incorporated into the GelNB bioink and printed with a DTT-free starch support material. The cytocompatibility of the indirect DTT supply strategy was confirmed across multiple cell types, and the dimensional stability and mechanical properties of the fabricated constructs were characterized.

Collectively, this work establishes starch slurry as a versatile and cell-friendly sacrificial support platform that can provide physical support while delivering distinct crosslinking agents to different functionalized gelatin bioinks. By eliminating the dependence of printability on the viscosity of the bioinks and minimizing the exposure of encapsulated cells to potentially cytotoxic crosslinkers, this strategy may expand the practical applicability of low-viscosity gelatin derivatives and offer an adaptable approach for fabricating cell-laden constructs for tissue engineering and regenerative medicine.

様式７

論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本論文において、Pekik Wiji Prasetyaningrum氏は、ゼラチン誘導体を用いる押出式3Dバイオプリンティングの造形性向上を目的として、造形物を物理的に支持すると同時に、支持材料－バイオインク界面から架橋剤を供給し、α-アミラーゼにより温和に除去可能なデンプン系犠牲支持材料を開発した。

まず、架橋反応の影響を排除した条件で、ゼラチンメタクリレート（GelMA）、フェノール性水酸基導入ゼラチン（GelPH）およびノルボルネン導入ゼラチン（GelNB）が細胞に及ぼす影響を比較した。線維芽細胞、子宮頸がん細胞、間葉系幹細胞および神経系細胞を用い、ミトコンドリア活性、増殖、形態、表現型関連遺伝子発現および骨分化を評価した結果、同程度の修飾率であっても、導入官能基に応じて細胞種依存的な応答が生じることを示し、バイオインク材料選択に有用な知見を得た。次に、過酸化水素を保持させた小麦デンプン支持材料と、西洋わさび由来ペルオキシダーゼを含むGelPHバイオインクを交互に押し出し、界面からの過酸化水素の拡散によってGelPHを架橋する造形法を確立した。間葉系幹細胞UE7T-13を用いた検討では、造形後の細胞生存性、幹細胞様特性および骨分化能が維持された。さらに、デンプン支持材料にジチオスレイトールを保持させ、GelNBへ界面から供給することで、細胞の架橋剤への直接曝露を抑えながら可視光によるチオールーエン架橋を行う方法へ展開した。複数種の細胞を用いて細胞適合性を確認するとともに、造形物の寸法安定性および機械特性を明らかにした。

以上の成果は、異なる架橋反応に対応し、物理的支持と架橋剤送達を兼ね備えたデンプン系犠牲支持材料の汎用性を示すものである。本研究は、低粘度ゼラチン誘導体の造形性向上と封入細胞の架橋剤曝露低減を両立する新たな方法論を提示し、組織工学および再生医療に向けた3Dバイオプリンティングの発展に寄与するものであり、博士（学術）の学位論文として価値のあるものと認める。