Preparation of MMP-sensitive Hyaluronic Acid Hydrogels and Their Impact on Cell Migration

*11229031 Shan Yu, Zhengwei Mao, Changyou Gao**

MOE Key Laboratory of Macromolecular Synthesis and Functionalization
Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China

Abstract:

Mimicking the structures and functions of extracellular matrix (ECM) is an important guidance for biomaterials design. ECM takes a key role in signal transduction. For example, enzymes are secreted or locally activated by migrating cells to degrade, and then to remodel the ECM. Therefore, a 3D matrix simulating the structure and function of ECM provides a better model system of physiological environment for cell migration study. Hyaluronic acid (HA) derivatives are widely used in biomedical field. The present study mainly focuses on the fabrication of an active and cell-responsive HA-based hydrogel. The migration of vascular smooth muscle cells (SMCs) is studied in the 3D matrix.

Introduction

Tissue engineering is a multidisciplinary field that aims to regulate tissue structure and function in vivo, and provide more physiologically relevant model systems for in vitro studies. [1] One of the basic element of tissue engineering is isolated cells or cell substitutes. The other is a proper extracellular environment for cells. Scaffolds or hydrogels can play the key role in tissue engineering by supporting chemical cues and mechanical integrity thus providing the adequate extracellular environment for cell distribution. [2]

The interactions between cells and materials can do effect the proper regeneration of tissues and organs, thus the conclusions summarized from in vitro and in vivo may guide the better design of materials. Cell migration plays a central role in many physiological processes, which is essential for proper immune response, wound repair, and tissue homeostasis, while aberrant cell migration is found in various pathologies. [3]

The present study mainly focuses on the fabrication of an active and cell-responsive HA-based hydrogel. The migration of vascular smooth muscle cells (SMCs) is studied in the 3D matrix.

Schematic

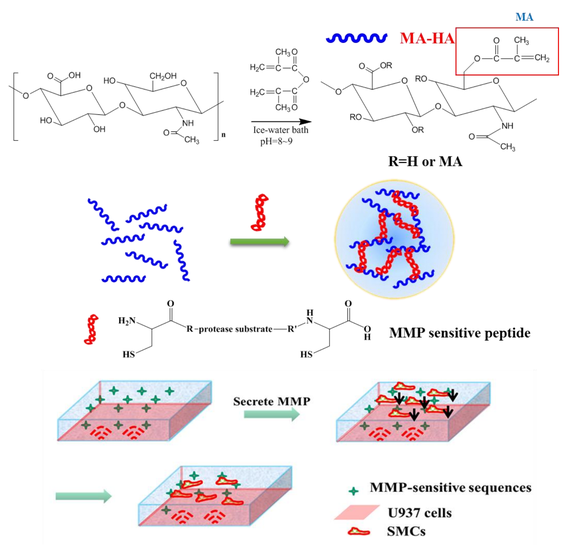

Fig. 1. Schematic illustration of fabrication of HA derivatives, formation of hydrogel, and the invasion of SMCs in a 3D matrix. The peptide (Ac-GCRD-GPQG-IWGQ-DRCG-NH_2, MMP SP) with two -SH groups and sensitive to matrix metalloproteinases (MMPs), a protease family extensively involved in tissue development and remodelling. U937 cells (a model macrophage cell) were used to be a signal source and MMP producer to induce the SMCs invasion to mimic the sequential tissue regeneration process in vivo. DTT crosslinked hydrogel was treated as a non-degradable hydrogel.

Mechanical Property

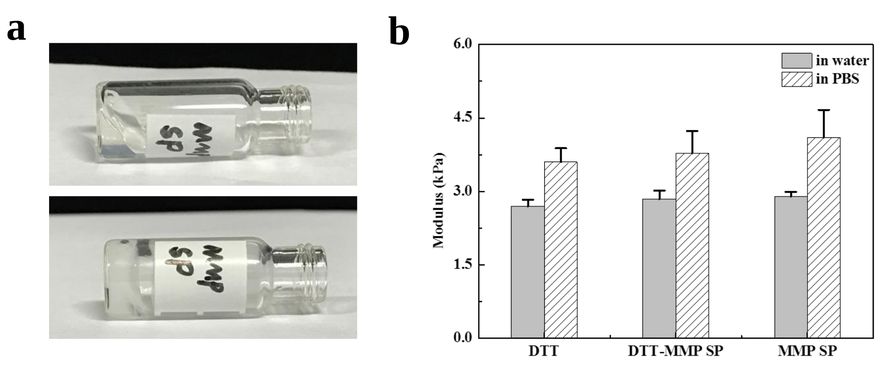

Fig.2. (a) Formation of MMP sensitive peptide (MMP SP) crosslinked hydrogel; (b) Mechanical properties of hydrogels in water and PBS.

Degradability

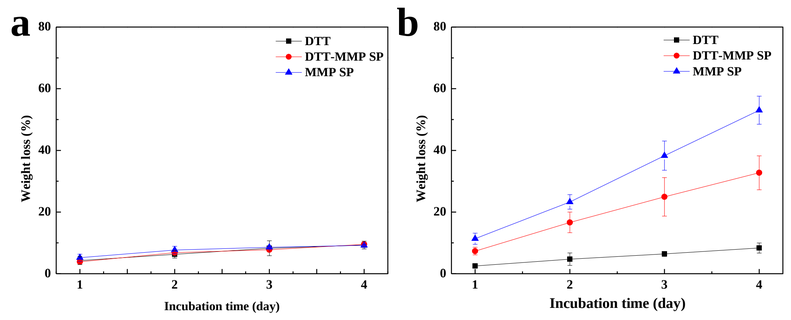

Fig. 3. (a) Weight loss of hydrogels in PBS; (b) Weight loss of hydrogels in PBS containing 0.2 μg/ml MMP.

3-Dimensional Invasion

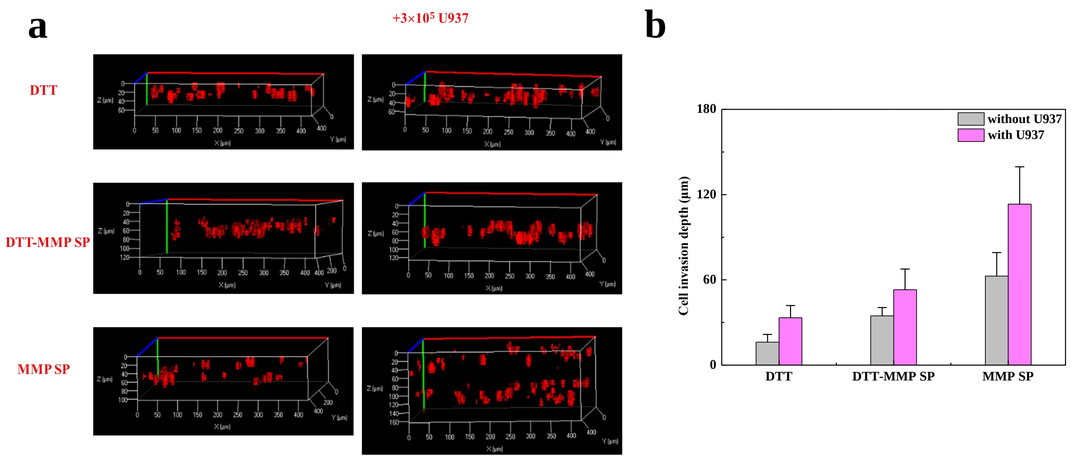

Fig. 4. (a) 3D constitution confocal images of SMCs invasion distance at 3 days in different hydrogels with or without U937 cells. (b) SMCs invasion distance at 3 days in different hydrogels with or without U937 cells.

In vivo characterization

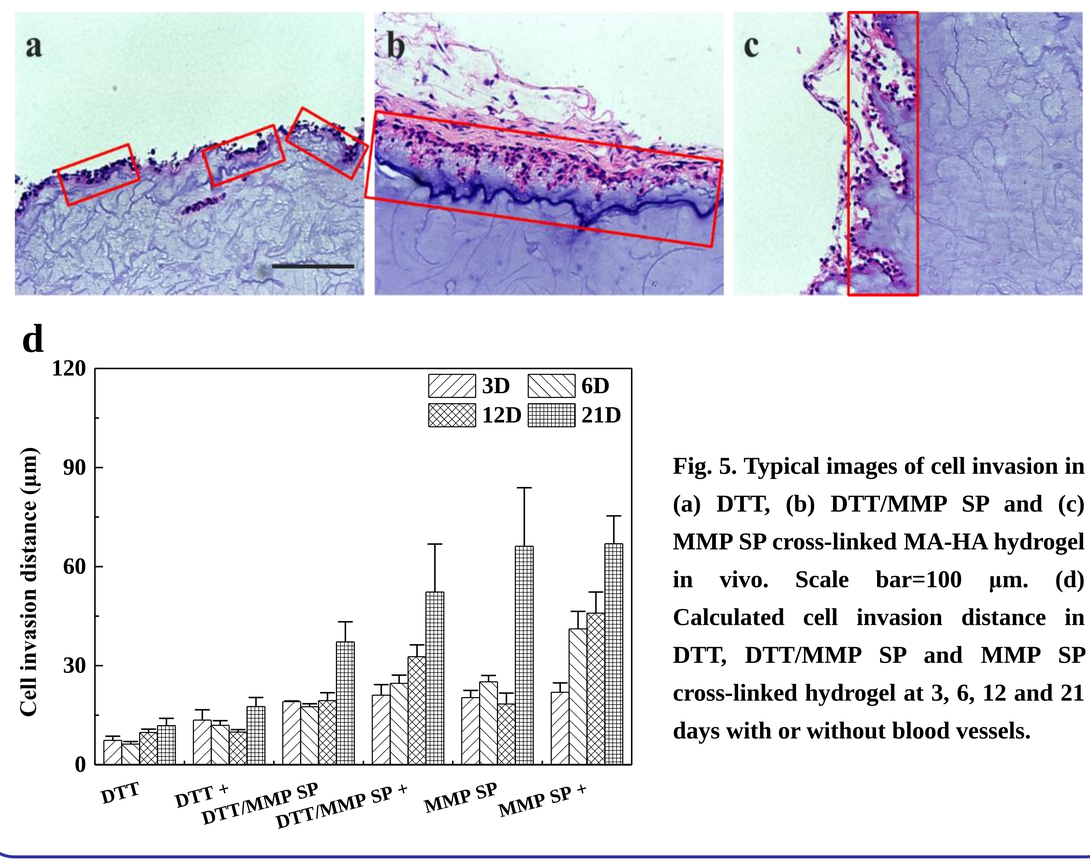

Fig. 5. Typical images of cell invasion in (a) DTT, (b) DTT/MMP SP and (c) MMP SP cross-linked MA-HA hydrogel in vivo. Scale bar=100 μm. (d) Calculated cell invasion distance in DTT, DTT/MMP SP and MMP SP cross-linked hydrogel at 3, 6, 12 and 21 days with or without blood vessels.

Conclusions

Through MMP SP crosslinking, a cell-responsive hydrogel was fabricated. In the non-degradable hydrogels, SMCs only invaded a distance of 50 μm into the hydrogels after 3 days, even with the attracting signal from U937 cells. In contrast, the SMCs invaded much deeper (over 150 μm) into the MMP-sensitive hydrogels toward the direction of U937 cells. The study provides a versatile model to study multiple important physiological processes, such as tissue regeneration and tumour metastasis. In vivo tests showed different invasion behaviors of three kinds of hydrogels and increased invasion distance as a function of time.

Acknowledgement

Financially support by the Natural Science Foundation of China (21434006 and 21374097), and the Key Science Technology Innovation Team of Zhejiang Province (2013TD02).

References

1. Leach, J.K. and D.J. Mooney, Synthetic extracellular matrices for tissue engineering. Pharmaceutical Research, 2008. 25(5): p. 1209-1211.
2. Deponti, D., et al., Collagen Scaffold for Cartilage Tissue Engineering: The Benefit of Fibrin Glue and the Proper Culture Time in an Infant Cartilage Model. Tissue Engineering Part A, 2014. 20(5-6): p. 1113-1126.
3. Trepat, X., Z.Z. Chen, and K. Jacobson, Cell Migration. Comprehensive Physiology, 2012. 2(4): p. 2369-2392.