Dear Dr. Lyndsay

I am very honored to be invited to publish paper on JoVE. I enclose a manuscript entitled "**Construction of multilayered stem cell sheet with a 3D dynamic culture system**", which I submit for possible publication in the **JoVE**. All of the authors agree to the submission of this paper.

In the past decade, cell sheet techniques have been rapidly developed, and exhibit advantages in cell therapy. However, an insufficient nutrition supply remains a major problem in maintaining stem cell viabilities in vitro. Considering that stem cell bioactivities are sensitive to the microenvironment, they will exhibit different behaviors according to varying cell sheet construction methods.

On the one hand, confluent cell sheets only consisted of high-density stem cells and natural extracellular matrices could be acquired by stacking monolayered cell sheets or using magnetic tissue engineering techniques. On the other hand, researchers developed different scaffolds to provide adequate mechanical strength and support cell growth, which allow low stem cell seeding density to ensure nutrition supply. However, despite these approaches, the low efficient nutrition supply within the multilayered cell sheet structure remains a major concern during the in vitro construction. Therefore, an efficient and feasible cell sheet construction system is urgently required.

The paper reports an efficient method for constructing a multilayered stem cell sheet. This cell sheet exhibits optimal mechanical strength, high cell seeding density, and favorable stem cell bioactivity. Using BMSCs as an example, the 3D cell structure is quickly constructed with RAD16-I peptide hydrogel. After being cultured in the dynamic perfusion system, the BMSCs multilayered cell sheet is successfully obtained and the BMSCs maintain high expression of stem cell markers.

The text includes 8 pages, 4 figures and a six-minute video according to the Instructions for Authors. We have provided all required supporting documentation.

We thank you for considering this work and look forward to your response.

Sincerely

Zheng Wu