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Jiankang Zhou#, Kun Zhang#, Shanshan Ma#, Tengfei Liu, Minghao Yao, Jingan Li*, Xiaofeng Wang and Fangxia Guan*

Preparing an injectable hydrogel with sodium alginate and Type I collagen to create better MSCs growth microenvironment

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Abstract: In the past few decades, stem cell transplantation has been generally accepted as an effective method on the treatment of tissue and organ injury. However, the insufficient number of transplanted stem cells and low survival rate that caused by series of negative conditions limit the therapeutic effect. In this contribution, we developed an injectable hydrogel composed of sodium alginate (SA) and Type I collagen (ColI), as the tissue scaffold to create better growth microenvironment for the stem cells. Compared the traditional SA scaffold, the ColI/SA hydrogel inherits its biomimetic properties, and simultaneously has shorter gelation time which means less loss of the transplanted stem cells. The mesenchyma stem cell (MSC) culture experiments indicated that the ColI/SA hydrogel could prevent the MSC apoptosis and contributed to faster MSC proliferation. It is highlighted that this ColI/SA hydrogel may have potential application for tissue regeneration and organ repair as the stem cell scaffold.

*** Corresponding authors: Jingan Li,** School of Material Science and Engineering, Zhengzhou University, 100 Science Road, Zhengzhou 450001, PR China, e-mail: lijingan@zzu.edu.cn **and Fangxia Guan**, School of Life Science, Zhengzhou University, 100 Science Road, Zhengzhou 450001, PR China; Center of stem cell and regenerative medicine, First Affiliated Hospital of Zhengzhou University, 40 University Road, Zhengzhou 450052, PR China, e-mail: fxguan@126.com.
Jiankang Zhou, Shanshan Ma and Tengfei Liu, School of Life Science, Zhengzhou University, 100 Science Road, Zhengzhou 450001, PR China.
Kun Zhang and Minghao Yao, School of Life Science, Zhengzhou University, 100 Science Road, Zhengzhou 450001, PR China; Center of stem cell and regenerative medicine, First Affiliated Hospital of Zhengzhou University, 40 University Road, Zhengzhou 450052, PR China.
Xiaofeng Wang, National Center for International Research of Micro-nano Molding Technology, Zhengzhou University, 100 Science Road, Zhengzhou 450001, PR China.
#Joint first authors.

Keywords: biomaterials; tissue regeneration; stem cell transplantation; scaffold; type I collagen

1 Introduction

Stem cell transplantation is generally accepted as one of the most effective methods for the clinical treatment of tissue and organ injury currently (1). However, stem cells loss and low cell viability after stem cell transplantation will count against better therapeutic effect (2). Therefore, it is essential to build an appropriate microenvironment for enhancing the therapeutic efficacy of transplanted stem cells. Tissue engineering scaffolds plays crucial roles on building the growth microenvironment (3-5), and preparing the tissue engineering scaffolds with series of molecules is a hot spot of research presently (6). This technology has many advantage, including degradability, appropriate water content and mechanical property, and good biocompatibility (7). In this work, two natural molecules, sodium alginate and Type I collagen, were chosen to prepare an injectable and biocompatible hydrogel as the tissue engineering scaffold to provide appropriate microenvironment for stem cells growth. Sodium alginate (SA) is a traditional material for the injectable hydrogel, and it can slow down the absorption of fatty sugar and bile salt, reduce the serum cholesterol, triglyceride and blood sugar, and prevent hypertension, diabetes, obesity and other diseases (8). Type I collagen (ColI) is a kind of polysaccharide protein, which contains a small amount of galactose and glucose, and it is the main component of the extracellular matrix that improves cell growth and migration (9,10). We hope this ColI/SA functional hydrogel as the tissue engineering scaffold may provide more potential application on tissue regeneration and organ repair.

2 Materials and methods

Preparation of the ColI/SA hydrogel: The ColI/SA hydrogel was prepared by the interaction of SA, $CaCO_3$, and ColI: In brief, SA (Sigma, USA) was dissolved in deionized water (dH_2O) to create 1% and 2% solution, while ColI (Sigma, USA) was dissolved in acetate buffer (1M) to create 0.5% solution (pH 7.2); Then the ColI and SA solution were mixed with concentration ratios of 1:4 (Samples were labeled as SA_4ColI_1) or 1:2 (Samples were labeled as SA_2ColI_1); Next, the $CaCO_3$ suspension and Gluconic acid lactone (GDL, Sigma-Aldrich) solution were introduced in the ColI/SA mixture to start the gel process (6); Finally, the ColI/SA hydrogels were obtained after 15 min reaction, and the single SA hydrogel prepared with the 2% solution was used as control in this study.

Characterization of ColI/SA hydrogel: The SA_4ColI_1, SA_2ColI_1 and SA hydrogels were photographed by a camera equipment to observe the whole object directly, and their morphology was observed by scanning electron microscopy (SEM, FEI quanta200, Netherlands) after freezing at -80°C, fully dried and gold spraying. The crystalline structure of each hydrogel was detected by an X-ray diffraction (XRD) characterization (4). The water contents, degradation performance, gelation time and rheological behavior of the hydrogels were also calculated as the previous work reported (8).

MSCs culture in the ColI/SA hydrogel: Mouse bone marrow mesenchymal stem cells (C57-170221I31) were purchased from Sayeye Biotech, China; the 3rd passage of MSCs was used to investigate the ColI/SA hydrogels' function on creating better MSCs growth microenvironment, including the MSCs distribution, density, proliferation and viability in the hydrogels (8,11,12).

3 Results and discussion

Figure 1a showed that all the hydrogels were molded into a cylindrical shape with the diameters of 15.6 mm after filled into the single hole of the 24-culture-plate and gelation. The SEM displayed their porous structures and the clusters alternately linked in a continuous network (Figure 1b). Notably, the SA, SA_4ColI_1 and SA_2ColI_1 hydrogels exhibited different pore diameters: The SA showed its pore diameters ranged from 300 μm to 1000 μm, while the SA_4ColI_1 presented its diameters from 200 μm to 500 μm, and the SA_2ColI_1 possessed the values from about 50 μm to 100 μm. The MSCs usually maintain their sizes in dozens of microns, thus SA_2ColI_1 may make more contribution for the MSCs loading and protection (13).

The XRD spectrum of SA, SA_4ColI_1 and SA_2ColI_1 hydrogels was presented in Figure 2. In case of SA hydrogel, a broad peak around $2\theta = 21°$ (characteristic amorphous peak) was observed which may be due to semi-crystalline structure of natural polysaccharide (SA). The small sharp

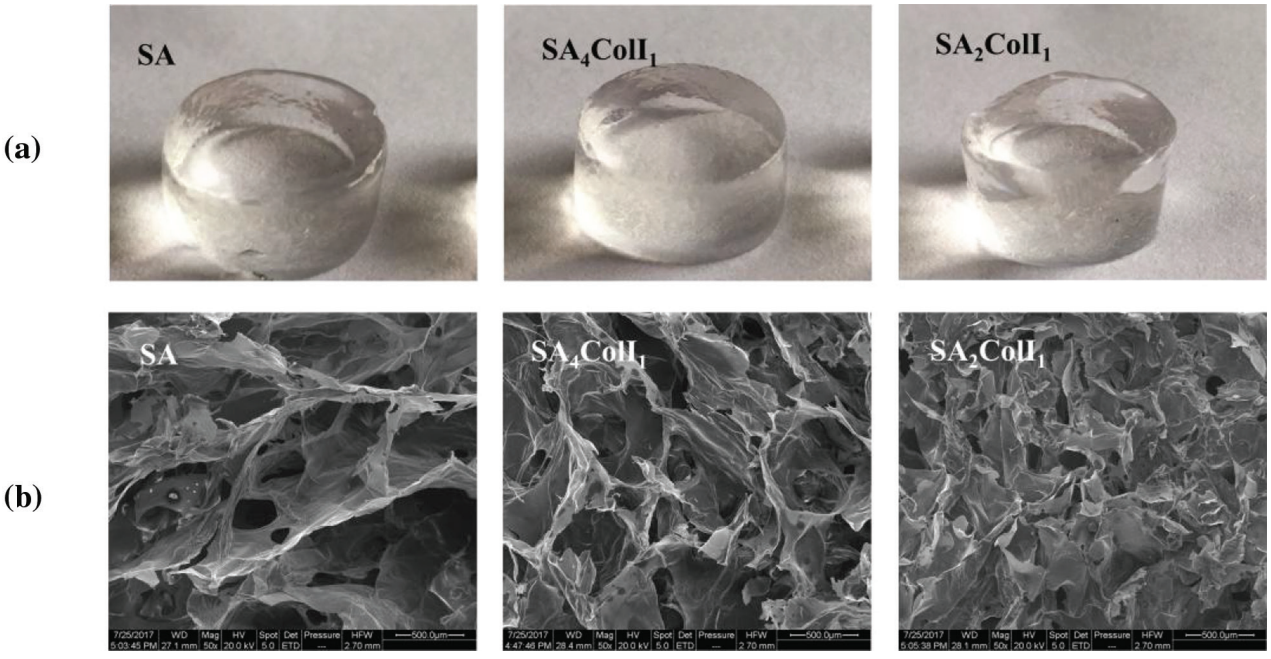

Figure 1: (a) The photographs and (b) microstructure of the SA, SA_4ColI_1 and SA_2ColI_1 hydrogels.

peaks at diffraction angle (2θ) of 29° was observed when the ColI was introduced to the hydrogels to be cross-linked as the SA_4ColI_1 and SA_2ColI_1 samples. The more ColI added, the stronger the sharper peaks presented, but the weaker the broad peak at 2θ = 21° became. The XRD results further demonstrated successful preparation of the SA, SA_4ColI_1 and SA_2ColI_1 hydrogels.

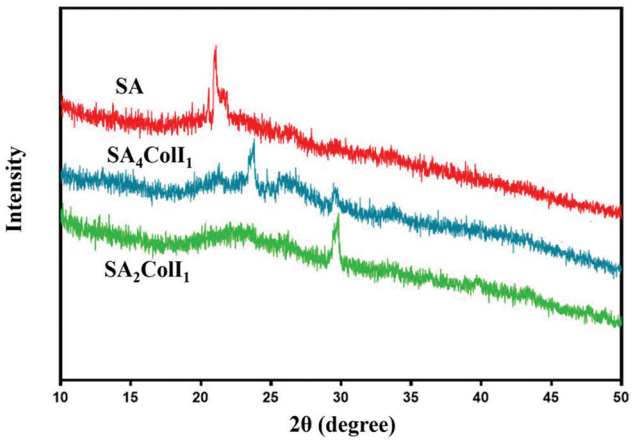

Figure 2: XRD spectra of the SA, SA_4ColI_1 and SA_2ColI_1 hydrogels.

Figure 3a showed that the gelation time of each hydrogel exceeded 5 min, and this time point is the requirements of the injectable implants for experimental and clinical application. The shorter gelation time surely provides better protection for the loaded cells and markedly reduced the cell loss in vivo. Thus, the gelation time result indicated that the SA_2ColI_1 may reduce the MSCs loss and provide better protection compared with SA_4ColI_1 and SA. The rheological behavior of the hydrogels was investigated *via* detecting their modulus of elasticity, and the modulus values of all the hydrogels ranged from 200 Pa to 1000 Pa (Figure 3b), which were similar to the human body's tissue modulus values, and this will contribute to the interaction of the implants and the focal tissue and improve the recovery of the damaged organs (14). Wherein, the SA_2ColI_1 presented a stable modulus values curve compared to SA_4ColI_1 and SA, which obviously made better matching degree for the tissues and may be more beneficial to the tissue repair and regeneration. All the hydrogels presented extremely high ratios of water content (higher than 95%, Figure 3c), creating a moist environment for cell loading and interaction, and the humid tissue scaffold also contributed to the factors

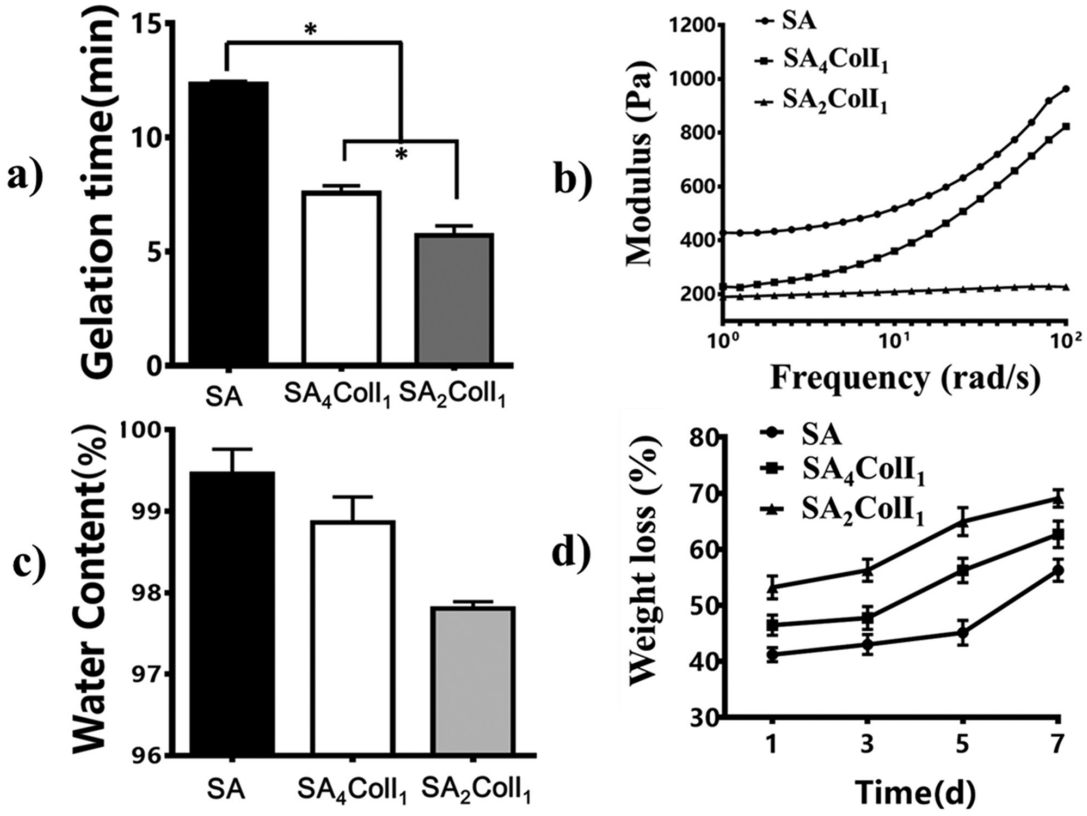

Figure 3: (a) Gelation time, (b) modulus, (c) water content, and weight loss of each hydrogel (mean ± SD, n=3, $*p < 0.05$).

exchange and cell migration between the implants and the focal tissue. Figure 3d displayed the degradation performance of the SA, SA_4ColI_1 and SA_2ColI_1 hydrogels: Although all the hydrogels showed rapid degradation speeds within 7 days, there is still at least 30% mass left to protect the loaded cells and the degradation speeds could be controlled by regulating the ColI ratio according to the different tissues' requirement.

Figure 4 showed the MSCs viability in each hydrogel: Obviously, the SA_2ColI_1 hydrogel exhibited higher viability ratio compared with SA and SA_4ColI_1 hydrogels, and this embodied in more green dots and less red dots in Figure 4a. More ColI and smaller pore diameters of SA_2ColI_1 hydrogel may be main contribution for the higher MSCs viability (8). In addition, the MSCs loading in the SA hydrogel presented a sharply declined viability ratio after the 5th days, and this period is the most crucial for the function recovery of the injury tissues, while the MSCs loading in the SA_2ColI_1 and SA_4ColI_1 hydrogels still maintained in the higher viability ratios within the first 7 days (> 80%) (Figure 4b), and this result also indirectly proved that the hydrogels degradation did not reduce the MSCs viability.

The CLSM was applied in a Z axis direction mode to investigate the MSCs proliferation and distribution in the SA, SA_4ColI_1 and SA_2ColI_1 hydrogels (Figure 5a), and the MSCs quantitative characterization were

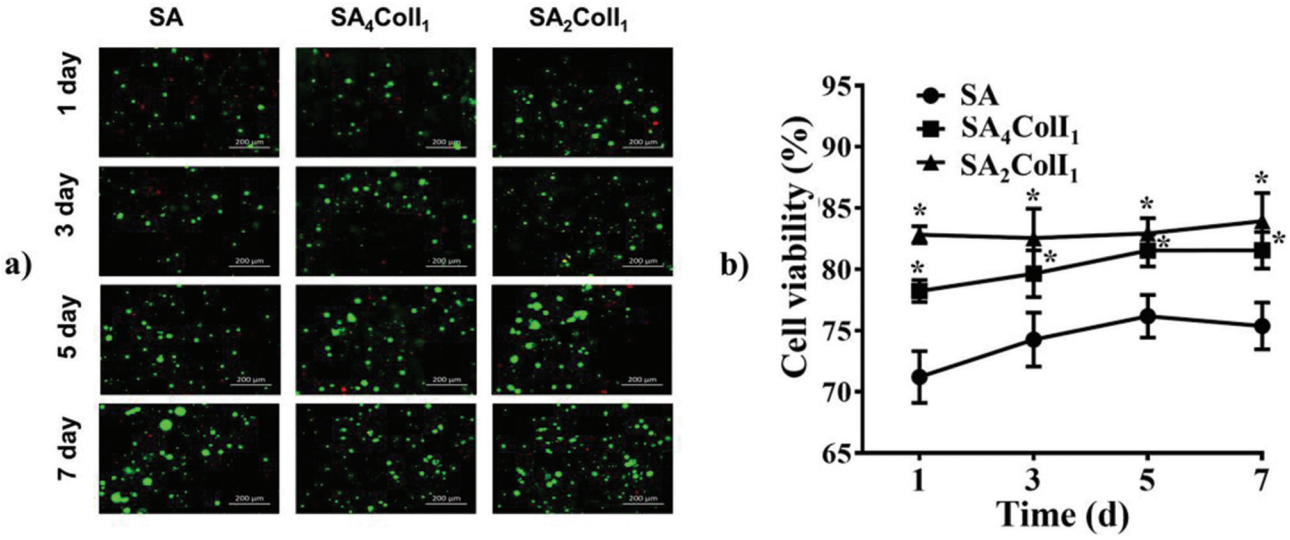

Figure 4: (a) Live/dead staining and (b) viability of MSCs loaded in each hydrogel (*p<0.05 compared with SA, mean ± SD, n=3).

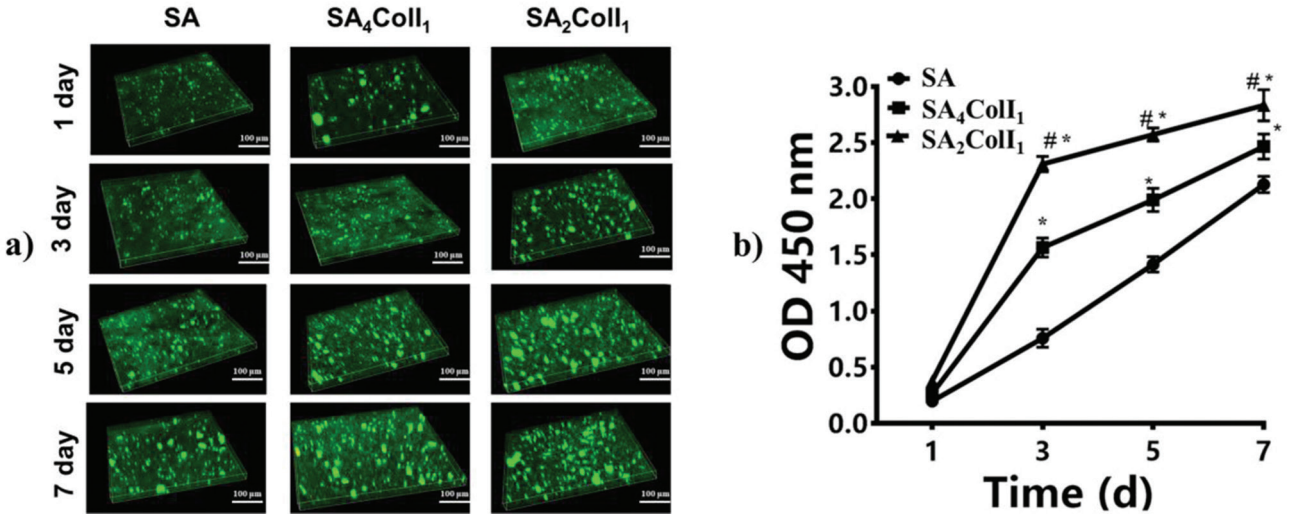

Figure 5: (a) CLSM images and (b) proliferation detection of MSCs distributed in each hydrogel (*p<0.001 compared with SA, $^{\#}p$<0.001 compared with SA_4ColI_1, mean ± SD, n=3).

also performed by a CCK-8 method (Figure 5b). From the CLSM images, MSCs homogeneously distributed in all the hydrogels, and the SA_2ColI_1 hydrogel showed higher MSCs density (more green dots) than the SA and SA_4ColI_1 hydrogels. The CCK-8 results also demonstrated that higher OD values (450 nm) appeared in the SA_2ColI_1 group at the 3rd day, 5th day, and 7th day although all the three curves displayed the upward trend, suggesting better ability on improving MSCs proliferation and activity.

4 Conclusions

In this contribution, we prepared an injectable hydrogel scaffold with Type I collagen (ColI) and sodium alginate (SA). The physical and chemical properties detection results indicated that the SA_2ColI_1 hydrogel had a shorter gelation time, which will provide better protection for the MSCs, and the SA_2ColI_1 hydrogel also showed more stable rheological behavior compared with the control, suggesting better contribution to the organization matching. All the hydrogels presented good water content and degradation performance. The living/dead staining results suggested that the MSCs in SA_2ColI_1 hydrogel possessed higher viability, and the CLSM images exhibited that the SA_2ColI_1 hydrogel displayed higher MSCs number and better proliferation compared to the controls. All these results suggest the feasibility of the SA_2ColI_1 hydrogel as a MSCs tissue engineering scaffold for the application on tissue repair and regeneration for its excellent ability to create better MSCs growth microenvironment.

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Conflicts of Interest: The authors declare no financial or commercial conflict of interest.

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