Off-the-shelf MSC-laden scaffold for enhancing tendon-bone interface healing in chronic rotator cuff injuries

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INTRODUCTION: There are over 75 million patients with chronic rotator cuff injuries globally. Among people over 60 seeking medical treatment for shoulder pain, rotator cuff injuries account for 60%. Elderly patients often choose conservative treatment, but improper treatment often leads to deterioration of the injury. This study aims to provide an economical and convenient treatment method.

METHODS: In this study, biological materials such as PLGA, BMP-2, TGF-β, and bFGF were used to prepare PLGA microspheres loaded with growth factors through a double emulsion technique. Using bone marrow mesenchymal stem cells (BMSCs) at a concentration of 2×10^7 cells/mL, three types of bioinks were formulated, and a three-dimensional tissue model simulating the tendon-bone interface was constructed through 3D bioprinting technology. To study the impact of cryopreservation on the performance of scaffolds, a cryoprotectant solution containing 1.5 mol/L DMSO, 0.1 mol/L fructose, 10 mmol/L Y27632, and 0.5 mmol/L ascorbic acid was used, and programmed freezing treatment was performed on the scaffolds. This study evaluated the impact of cryopreservation on the viability of scaffold cells and the characteristics of stem cells, and compared the biomechanical properties of frozen and fresh scaffolds. Through a rat rotator cuff tear model, the in vivo repair effect of bioprinted constructs was further evaluated, providing a potential scientific basis for clinical treatment.

RESULTS: In the cell viability assessment, through calcein/PI double staining, TUNEL staining, scanning electron microscopy observation, and biomechanical testing, there was no significant difference in the number of living cells and the percentage of apoptotic cells between the frozen scaffold group and the fresh scaffold group ($P>0.05$). Stem cell characteristic analysis showed that the colony formation ability, proliferation ability, and multidirectional differentiation ability of the two groups were similar ($P>0.05$). In the evaluation of mechanical properties, no significant difference in the cross-sectional area of the supraspinatus muscle was found among the groups at 4 and 8 weeks after surgery. At 4 weeks after surgery, the ultimate load failure and stiffness exhibited by the frozen scaffold group were higher than those of the control group and were comparable to those of the fresh scaffold group. At 8 weeks after surgery, the ultimate load failures of the control group, fresh scaffold group, and frozen scaffold group recovered to 68.22%, 85.23%, and 83.67% of the normal group, respectively, showing a gradual improvement in repair effects. In the rat rotator cuff tear model, at 4 weeks after surgery, the control group showed more inflammatory cells and fibrovascular tissues, while the frozen scaffold group and fresh scaffold group had less inflammatory response, and there was no difference between the two groups. At 8 weeks after surgery, the frozen scaffold group was superior to the control group in fibrocartilage area regeneration and tendon thickness, and there was no significant difference in tendon repair status between the frozen scaffold group and fresh scaffold group.

DISCUSSION AND CONCLUSION: The bio-cell printed scaffold preserved by programmed freezing has the same therapeutic effect as the fresh scaffold. This freezing preservation method is effective, improves the cell survival rate after scaffold resuscitation, reduces scar tissue formation, provides more possibilities for clinical treatment, and is expected to improve the treatment prognosis and quality of life of elderly patients.