http://jmscr.igmpublication.org/home/
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v13i08.03

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

Viability of Mesenchymal Stem Cells in Commercial Fibrin Glue: Pilot study

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Abstract

Purpose: *Rotator cuff tear is a common injury that often requires surgical intervention due to poor healing capacity and high chances of retear. While surgical repair helps regain function, its long-term outcome is discrepant. Recently, mesenchymal stem cells (MSCs) have been explored as an adjunct therapy in rotator cuff repair. Studies suggest they may improve tendon integrity and reduce retear rates. To enhance MSC retention and viability at the repair site, carriers like fibrin glue have been explored. Fibrin glue provides a three-dimensional scaffold that mimics the final stage of clot formation and supports cell survival and proliferation. However, clinical studies on MSCs combined with commercial fibrin glue have reported contradictory results, with some showing clinical benefits and others indicating no significant improvement in clinical outcomes. This study was carried out to assess the viability of MSCs in commercial fibrin glue.*

Materials and Methods: *MSC viability in commercial fibrin glue over a period of one-hour was compared to MSCs in suspension.*

Results: *The results showed an immediate drop in cell viability within the fibrin glue group to 74.0% ± 2.00 at time 0, whereas the control group maintained a higher viability of 98.3% ± 0.58. This was followed by a gradual decline in both groups, with a comparable rate of decrease over time.*

Conclusion: *These findings indicate that modifications in fibrin glue composition may be necessary to improve MSC survival and optimize regenerative outcomes in rotator cuff repair.*

Keywords: *Mesenchymal stem cells; Fibrin glue; Viability assay; Rotator cuff tear.*

1. Introduction

Rotator cuff tears is one of the most prevalent shoulder diseases, affecting over 50% of individuals above 60 years of age. Current treatment options include physical therapy and surgical repair. However, surgical procedures are often times challenging and associated with high chances of retear (11–95%), hence demanding improved treatment strategies[1].

One promising avenue of treatment is with mesenchymal stem cell (MSC) therapy. MSCs possess self-renewal capabilities and multilineage differentiation potential**,** making them ideal candidates for tissue regeneration[2]. Their ability to migrate to injury sites via chemotaxis, develop into mesodermal tissues, including bone, muscle, ligament, cartilage and tendon[3] makes them particularly suited for rotator cuff repair[4]. Several preclinical and clinical studies that have employed the use of MSCs in the treatment of rotator cuff injuries have given promising outcomes, though the research on it is ongoing and nascent[1].

Mesenchymal stem cells are transplanted into the body using biomaterials as carriers. Among biomaterials such as collagen and hyaluronic acid**,** fibrin glue is a widely used. In orthopedic procedures, fibrin glue is commonly used as a sealant to control bleeding or create fluid-tight closures[5]. However, fibrin glue's effectiveness as an MSC carrier remains uncertain. Some studies suggest that fibrin glue enhances bone regeneration and tendon healing[6], while others report no significant clinical benefits[7,8]. Given the conflicting results regarding the use of fibrin glue as an MSC carrier in rotator cuff repair, this study aimed to evaluate the viability of MSCs within commercial fibrin glue (Tisseel) over a one-hour period and provide insights into the suitability of fibrin glue as a scaffold for MSC-based regenerative therapies in rotator cuff repair.

2. Materials And Methods

2.1. Preparation of mesenchymal stem cells:

The mesenchymal stem cells of passage no.5 were obtained from Stempeutics Research Pvt. Ltd. (Bangalore, India). The cell suspension was centrifuged at 300 × g for 5 minutes at room temperature to form pellets of 25 million cells. Following centrifugation, the supernatant was carefully aspirated without disturbing the cell pellet.

2.2. Preparation of Fibrin Glue:

The fibrin glue used in this study was Tisseel 2 mL fibrin sealant kit (Baxter, USA). The components present in the kit were pre-warmed to the recommended temperature of 37°C in a water bath for optimal functionality. The fibrinogen and thrombin solutions were reconstituted according to the manufacturers instructions and the solutions were transferred at full concentrations into separate sterile syringes given with the kit. For mixing with stem cells, the cells were suspended in the fibrinogen solution first. The fibrinogen-cell suspension syringe and the thrombin syringe were then connected to the dual-barrel applicator. The fibrin glue was prepared and kept ready just before the procedure to prevent premature polymerization and ensure maximum efficacy.

2.3. Mixing Stem Cells with Fibrin Glue:

The cell-fibrinogen mixture and thrombin solution were injected into a sterile tube using the dual-barrel applicator. The contents were gently mixed using a tip attached to a pipette, to ensure that the stem cells were evenly distributed within the fibrin glue without causing premature polymerization.

2.4. Cell viability assay:

Cell viability was assessed using the Thermofisher Countess 3FL automatic cell counting machine at 5 time points- 0 minutes, 10 minutes, 30 minutes, 45 minutes and 1 hour after preparation. At each time point, a small aliquot of the cell-fibrinogen-thrombin mixture was taken and mixed in equal ratio with the viability staining dye Tryan blue, then 10µL of this mix was taken for cell counting. This procedure ensured that the effect of the fibrin glue mixture on cell viability was carefully monitored over time. Periodically, the cells were also counted manually to reaffirm the values. Each experiment was performed three times independently to ensure reproducibility, and the mean viability values were calculated from these replicates.

2.5. Statistical analysis:

The experiments were conducted in triplicate, and results are expressed as mean ± standard deviation. A paired t-test was used to compare viability percentages between the control and fibrin glue groups at each time point, with $P < 0.05$ considered as statistically significant. To quantify the rate of decline in cell viability over time, the slope of the graphs was calculated using the formula: $m= (y2 -y1)/(x2-x1)$, where m is the slope and represents the rate of change of viability percentage per minute, y2 and y1 are the mean viability percentages at time points x1 and x2 respectively.

3. Results

Mesenchymal stem cell viability was assessed over a period of one hour in control conditions (only cells) and with fibrin glue. While the control group maintained high viability, there was sharp initial decline in the viability of cells seeded with fibrin glue.

At time 0, the mean viability of MSCs in the control group was 98.3% ± 0.58, while in fibrin glue, it was significantly lower at 74.0% ± 2.00. A progressive decline in viability was observed in the fibrin group over time, dropping to 71.7% ± 1.53 at 10 minutes, 70.3% ± 2.08 at 30 minutes**,** 68.7% ± 0.58 at 45 minutes, and 68.0% ± 1.00 at 1 hour.

In contrast, the control group exhibited minimal reduction in viability, maintaining 97.7% ± 0.58 at 10 minutes**,** 96.7% ± 0.58 at 30 minutes**,** 95.3% ± 0.58 at 45 minutes, and 93.3% ± 1.15 at 1 hour. The difference in viability between the two groups remained statistically significant across all time points ($P < 0.001$).

Table 1. MSC Viability (%) in Control vs. Fibrin Glue Over Time

Table 1 summarizes the statistical findings, highlighting the significant reduction in viability of MSCs in fibrin glue compared to the control.

Time (minutes)	Control viability (%)	Fibrin glue viability (%)
0	98.3 ± 0.58	74.0 ± 2.00
10	97.7 ± 0.58	71.7 ± 1.53
30	96.7 ± 0.58	70.3 ± 2.08
45	95.3 ± 0.58	68.7 ± 0.58
60	93.3 ± 1.15	68.0 ± 1.00

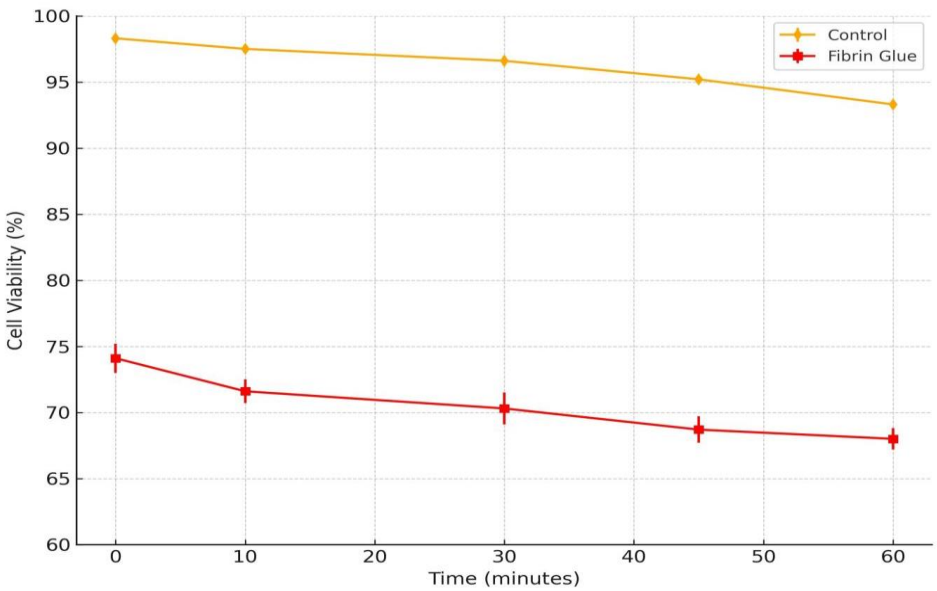

Fig. 1. MSC Viability Trend in Control vs. Fibrin Glue Over Time

The data is further visualized in **Fig. 1**, which depicts the mean viability percentages with standard deviation error bars.

Further, using the data from 0 to 60 minutes, rate of decline of cell viability for both the control and fibrin glue groups were calculated. The fibrin group starts at a much lower viability (74%) compared to the control (98.33%), indicating an immediate cell loss (around 25% difference). After the initial drop, the control group exhibited a decline in cell viability at a rate of −0.0833% per minute, while the fibrin glue group declined at −0.1% per minute. Although this difference in slope is statistically significant (P = 0.023), it is only minor compared to the intial drop. This suggests that following an initial sharp decrease of approximately 25% in cell viability within the fibrin glue group, both groups experienced a similar gradual decline, with only a slightly faster rate observed in the fibrin glue group.

4. Discussion

This study assessed the short-term viability of MSCs in commercial fibrin glue (Tisseel) over a 1-hour period compared to MSCs in suspension. Results showed a significant initial drop in viability upon fibrin glue exposure, likely due to an unfavorable microenvironment during polymerization. However, the subsequent decline was modest and comparable to the control group. At time 0, MSC viability in the fibrin glue group was 74.0% ± 2.00, significantly lower than the 98.3% ± 0.58 observed in the control group (P < 0.001). This ~25% reduction in viability points toward an acute stress response following fibrin polymerization[6]. Nonetheless, beyond the initial drop, MSC viability declined only slightly over time, from 74.0% at time 0 to 68.0% at 1 hour, whereas the control group decreased from 98.3% to 93.3%. To quantify this trend, the slopes of the viability curves were calculated as -0.0833% per minute for control group and -0.1% per minute for fibrin glue group. The difference in slopes between the two groups is statistically significant (P=0.023), however it is minimal compared to the initital drop of ~25% in viability, suggesting that once the cells in fibrin glue survive the initial polymerization process, the subsequent decline in viability occurs at a rate comparable to cells in suspension. This observation indicates that the primary detrimental effect of fibrin glue occurs at the moment of polymerization, with relatively minor viability loss afterward. One of the likely contributors to this early viability loss is the composition of commercial fibrin glue. Unlike physiological fibrin, which contains 2–4 mg/mL of fibrinogen, commercial fibrin glues like Tisseel contains 67–106 mg/mL fibrinogen—about 30 times higher than in native plasma. While this concentration facilitates rapid clot formation for hemostatic purposes, it may simultaneously limit oxygen and nutrient diffusion, thereby reducing cell survival. Additionally, high thrombin levels (>500 IU/mL) present in Tisseel, have been implicated in cytotoxic effects on MSCs in other studies [9, 10]. Commercial fibrin glue is advised to be used only as as a sealant or adjunct with mesenchymal stem cells in clinical procedures. Its efficacy as a matrix to seed and help proliferate mesenchymal stem cells is not confirmed. According to Tisseel fibrin glue datasheet, it is to be used in a similar manner and its effect on lab tests has not been established [11]. However, clinical procedures often utilise injectible scaffolds that add the mesenchymal stem cells with one of the components of the commercial fibrin glue kit , e.g, in CartiFill scaffold (Sewon Cellontech, Seoul, Korea) for arthroscopic Cartilage repair treatment, CartiFill collagen implant is mixed with the thrombin solution before it is mixed with the fibrinogen solution for polymerisation[12]. The results of this study align with prior research demonstrating variability in the effectiveness of fibrin glue as an MSC carrier. Some studies have suggested that fibrin glue can enhance MSC retention and differentiation, such as in bone and tendon regeneration[6], while others have reported no significant benefits[7]. While variations in fibrin glue formulations are a key factor, discrepancies in outcomes across studies may also be influenced by differences in MSC sources and experimental conditions[13]. Clinically, these findings suggest that the use of commercial fibrin glue as a scaffold for MSC-based therapies for rotator cuff repair should be approached with caution. While commercial fibrin glue is well-established as a surgical sealant and delivery aid, its potential to support MSC survival and proliferation remains

uncertain based on these early results. Further research—including longer-term studies and in vivo assessments—is essential to fully understand its suitability as a scaffold in MSC-based therapies for rotator cuff repair.

5. Limitations

This study presents preliminary findings and, as such, is subject to several limitations. First, the experiments were conducted using a single commercial fibrin glue formulation (Tisseel), and the results may not be generalizable to other products. Second, only short-term viability was assessed over a one-hour period. While this time frame highlights the immediate impact of fibrin polymerization on MSCs, it does not provide information about long-term cell survival, proliferation, or functional behavior within the matrix. A more comprehensive assessment, including proliferation assays and in vivo studies, would help clarify the biological impact of fibrin glue on MSC behavior. Third, the study was conducted in vitro under controlled conditions, which may not fully replicate the complex mechanical and biochemical environment of in-vivo rotator cuff repair. Lastly, a larger sample size and broader biological replicates would enhance statistical power and confidence in the results.

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