Effects of Commercial vs. Autologous Fibrin Glues on Chondrocyte Viability & Gene Expression

Apratim Maity, Jackson J Hubbell, Pooja Swami, John G Lane, Daniel A Grande

INTRODUCTION:

Matrix-induced autologous chondrocyte implantation (MACI) is considered the gold standard for surgical treatment of post-traumatic osteoarthritis (PTOA) of the knee. In MACI, fibrin glue is used to secure a chondrocyte-laden collagen membrane and promote cartilage repair. However, concerns about the efficacy of commercial fibrin glues in supporting chondrocyte migration and proliferation have prompted further investigation into its biological effects and potential autologous alternatives[1]. Cryoprecipitate, a blood product derived from the slow thawing of fresh frozen plasma, contains fibrinogen, which, when combined with the enzyme thrombin and $CaCl_2$, can produce a rich fibrin clot that can be used as a fibrin glue substitute. We hypothesize that using a cryoprecipitate-derived fibrin glue may impart superior chondrogenic effects on the final MACI autograft compared to commercially produced glue. Furthermore, platelet-rich plasma (PRP), which is rich in growth factors, has emerged as an adjunct treatment to modulate inflammation and promote a healing environment, so infusing such a glue with PRP may also shift the local cellular milieu from pro-inflammatory to anti-inflammatory[2]. This study evaluated the effects of cryoprecipitate-derived (CT), PRP-infused cryoprecipitate-derived (CTP) and commercial (COMM) fibrin glues on chondrocyte viability and gene expression.

METHODS: 200,000 human chondrocytes were seeded onto a 24-well plate and incubated for 24 hours to allow for cell attachment. On the following day, fibrin glues were dispensed into empty Petri dishes and allowed to solidify. A 10-mm biopsy punch was then used to extract uniformly sized glue discs for application. CT glue was prepared by combining bovine thrombin and $CaCl_2$ with cryoprecipitate derived from human donor blood. For CTP glue, PRP was isolated and incorporated into the CT mixture. CT, CTP, or COMM glues were applied to chondrocyte-seeded wells of a 24-well plate, ensuring direct contact between the glue and cells. A no-glue control was included as a comparator. Gene expression of ACAN, MMP-13, TGF-β, and Bcl-2 was assessed on Days 1, 3, and 7 via qPCR. Calcein-AM/Ethidium Homodimer Live/Dead staining was performed on Days 1 and 3.

RESULTS: On Day 1, CT and CTP groups exhibited significantly reduced MMP-13 expression (CT: $p = 0.0070$; CTP: $p = 0.0179$). On Day 3, COMM showed decreased ACAN expression ($p = 0.0388$). By Day 7, MMP-13 expression was significantly increased in the COMM group ($p = 0.0478$). No significant differences were observed in Bcl-2, TGF-β, or chondrocyte viability.

DISCUSSION AND CONCLUSION:

Successful early MACI graft integration is critical for long-term cartilage repair[3]. Reduced MMP-13 expression observed on Day 1 in CT and CTP groups may reflect a more favorable early remodeling environment, as excessive MMP-13 activity contributes to matrix degradation and may impair graft stability. Conversely, the decreased ACAN expression in the COMM group on Day 3 raises concern, as aggrecan is essential for cartilage integrity and load-bearing capacity[4]. Notably, the COMM group also showed a significant increase in MMP-13 expression on Day 7, suggesting catabolic activity that could further compromise cartilage matrix integrity. Additionally, no significant differences were observed in Bcl-2 expression or cell viability across groups, indicating that none of the fibrin glues tested, including the commercial fibrin glue, were cytotoxic under the conditions studied. While these findings suggest that fibrin glue does not cause noticeable cell death, they may still influence gene expression patterns relevant to graft integration and cartilage repair. Collectively, these results highlight important biological differences between fibrin glues and support continued investigation into their influence on MACI outcomes.

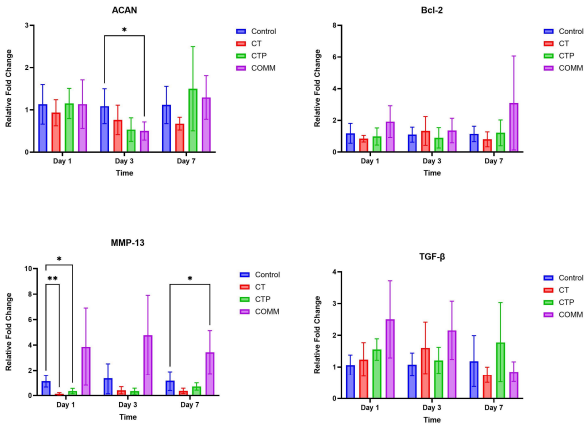
References:

1. Migliorini F, Prinz J, Maffulli N, Eschweiler J, Weber C, Lecoutrier S, et al. Fibrin glue does not assist migration and proliferation of chondrocytes in collagenic membranes: an in vitro study. J Orthop Surg Res. 2022;17:311. doi:10.1186/s13018-022-03201-6.

2. Li T, Li Y, Li W, Wang X, Ding Q, Gao J, et al. Impact of autologous platelet-rich plasma therapy vs. hyaluronic acid on synovial fluid biomarkers in knee osteoarthritis: a randomized controlled clinical trial. Front Med (Lausanne). 2023;10:1258727. doi:10.3389/fmed.2023.1258727.

3. Dunkin BS, Lattermann C. New and emerging techniques in cartilage repair: MACI. Oper Tech Sports Med. 2013;21(2):100–7. doi:10.1053/j.otsm.2013.03.003. PMID: 24072960; PMCID: PMC3780415.

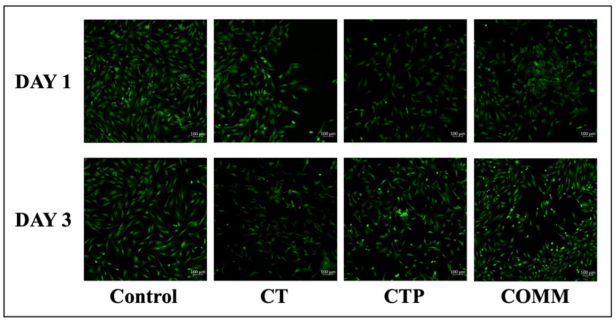
4. Kiani C, Chen L, Wu Y, Yee AJ, Yang BB. Structure and function of aggrecan. Cell Res. 2002;12(1):19–32. doi:10.1038/sj.cr.7290106.


ACAN
Relative Fold Change
Time
Day 1
Day 3
Day 7
Control
CT
CTP
COMM
*

Bcl-2
Relative Fold Change
Time
Day 1
Day 3
Day 7
Control
CT
CTP
COMM

MMP-13
Relative Fold Change
Time
Day 1
Day 3
Day 7
Control
CT
CTP
COMM
*
**
*

TGF-β
Relative Fold Change
Time
Day 1
Day 3
Day 7
Control
CT
CTP
COMM


DAY 1
DAY 3
Control
CT
CTP
COMM