A Proof-of-Concept Study to Evaluate the Clinical Study Safety and Efficacy of Ex-vivo Cultivated Allogeneic Limbal Stromal Stem Cell Transplantation for Treatment of Superficial Corneal Pathologies after Alginate Encapsulation: Two years Follow up

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PURPOSE

This study proposes to investigate the transplantation of human limbus-derived mesenchymal stem cells (hLMSCs) for the treatment of the corneal pathologies after alginate encapsulation. We previously showed the preclinical safety and efficacy of hLMSCs and successfully completed a phase 1 clinical trial (CTRI/2021/07/035034). Further, these cells are able to successfully engraft, differentiate, and mediate wound healing in the corneal stroma such that the tissue remains healthy, free of fibrotic tissue, and optically transparent.

METHODS

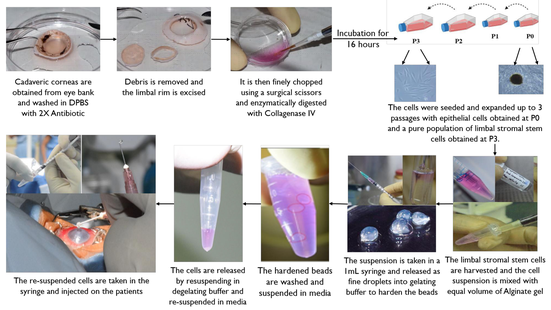


Figure 1 : Representative Image of the Work Flow of the Culture, Encapsulation and Delivery

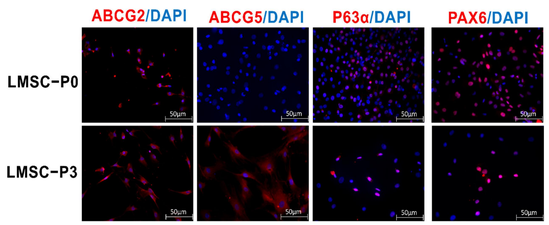


Figure 2: Expression of the stem cell and ocular biomarkers in limbal stem cells. Representative images of the limbal stem cells showing positive expression of ABCG2, ABCB5, P63-α, and PAX6 (red) in both epithelial (LMSC−P0) and stromal cell (LMSC−P3) populations, counterstained with DAPI (blue). Scale: 50 µm.

- This study was interventional, single arm open labelled Phase I clinical trial (CTRI/2020/07/026891)
- The subject inclusion criteria were adult patients with superficial corneal pathologies such as scars, sterile ulcers and burns.
- Exclusion criteria included bilateral corneal disease and dry eye disease.
- The treatment procedure involved excision of corneal epithelium followed by topical application of cells mixed with fibrin glue.
- The primary endpoint was safety evaluation and the secondary endpoint were efficacy parameters such as visual improvement and change in density of scar and other pathologies.
- The study enrolled a total of 20 participants. The age distribution among the subjects showed a mean (± standard deviation) of 39.4 ± 11.16 years, indicating a moderately wide age range across the sample.
- The median age was 40.5 years, with the interquartile range (Q1, Q3) spanning from 29.5 to 49.0 years, suggesting that half of the participants were within this age bracket. The minimum and maximum ages were 21.0 and 60.0 years, respectively

RESULTS

Sl. No	Subject ID	Age	Sex	Diagnosis	Remarks
1	2R01	35 years	Male	Corneal scar after microbial keratitis	No safety issues.
2	2R02	32 years	Male	Corneal scar after microbial keratitis	No safety issues.
3	2R03	54 years	Male	Corneal scar after microbial keratitis	No safety issues.
4	2R04	42 years	Female	Corneal scar after microbial keratitis	No safety issues.
5	2R05	21 years	Male	Chemical injury (Burns)	No safety issues.
6	2R06	28 years	Male	Corneal scar after microbial keratitis	No safety issues.
7	2R07	28 years	Male	Chemical injury (Burns)	No safety issues.
8	2R08	39 years	Male	Corneal scar after microbial keratitis	No safety issues.
9	2R09	48 years	Male	Corneal scar after microbial keratitis	No safety issues.
10	2R10	43 years	Male	Corneal scar after microbial keratitis	No safety issues.
11	2R11	25 years	Male	Corneal scar after microbial keratitis	No safety issues.
12	2R12	60 years	Male	Corneal scar after microbial keratitis	No safety issues
13	2R13	46 years	Female	Corneal scar after microbial keratitis	No safety issues
14	2R14	51 years	Male	Corneal scar after microbial keratitis	No safety issues
15	2R15	42 years	Male	Corneal scar after microbial keratitis	Delayed Epithelialization
16	2R16	31 years	Male	Corneal scar after microbial keratitis	No safety issues
17	2R17	46 years	Female	Corneal scar after microbial keratitis	Delayed Epithelialization
18	2R18	27 years	Male	Corneal scar after microbial keratitis	No safety issues
19	2R19	31 years	Female	Corneal scar after microbial keratitis	Delayed Epithelialization
20	2R20	51 years	Male	Corneal scar after microbial keratitis	No safety issues

Table 1: Summary of Demographics.

Clinical Outcomes after hLMSC Application

Figure 3: Long-term outcomes of allogeneic limbal stromal stem cell transplantation. Slit-lamp, AS-OCT, and Scheimpflug images (left to right) show progressive corneal recovery from pre-operative showing corneal scare and edema representative cases. All the cases show restored transparency and thickness at 1 and 2 year follow-ups, indicating sustained graft integration and structural improvement.

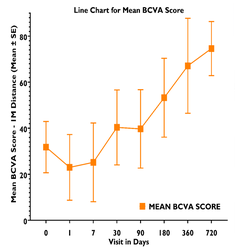


Figure 4: Line Chart for Mean (±SD) BCVA Score – Total No. of Letters read at 1M and 4M Distance by the Operated Eye

Statistics	Baseline	Day 1	Day 7	Day 30	Day 90	Day 180	Day 360	Day 720
Sample size (n)	20	13	18	20	17	16	19	18
Mean ± SD	31.80 ± 11.15	23 ± 14.27	25.17 ± 17.08	40.35 ± 16.28	39.71± 17.02	53.25 ± 17.14	67.11 ± 20.64	74.61 ± 11.74
Median	33	20	30	41.50	39	52	68	78
(Q1, Q3)	25, 40.50	10, 35	05, 40	30, 51	29, 50	40, 62	59, 85	67, 85
Min, Max	0.0, 43	0.0, 45	0.0, 50	5.0, 75	10, 75	24, 84	0.0, 90	45, 87
p value		0.0091	0.0792	0.0426	0.0469	0.0002	0.0000	0.0001

Table 2: Overall Summary of BCVA in Operative Eye. SD: Standard Deviation, Min: Minimum; Max: Maximum; Q1: First Quartile; Q3; Third Quartile; CI: Confidence Interval; Change from baseline = Post values–Baseline values; Statistical Method: Paired t test; p value : considered Screening Period as the baseline; p-value <0.05 is significant.

Statistics	Baseline	Day 1	Day 7	Day 30	Day 90	Day 180	Day 360	Day 720
Sample size (n)	19	12	16	19	16	16	18	17
Mean ± SD	0.67± 0.39	0.55± 0.39	0.23± 0.42	-0.11± 0.26	-0.24± 0.25	-0.19± 0.42	-0.43± 0.39	-0.32± 0.60
Median	0.60	0.53	0.20	-0.10	-0.20	-0.20	-0.31	-0.30
(Q1, Q3)	0.30, 1.00	0.31, 0.91	0.00, 0.31	-0.20, 0.10	-0.46, -0.04	-0.41, -0.14	-0.72, -0.18	-0.80, -0.18
Min, Max	0.18, 1.40	-0.10, 1.12	-0.50, 1.42	-0.70, 0.30	-0.70, 0.20	-0.72, 0.82	-1.10, 0.30	-1.10, 0.82
p value		0.0005	0.0509	0.0803	0.0016	0.0904	0.0002	0.0438

Table 3: Summary of Overall Snellen LogMar.
SD: Standard Deviation, Min: Minimum; Max: Maximum; Q1: First Quartile; Q3; Third Quartile; CI: Confidence Interval; Change from baseline = Post values–Baseline values; Statistical Method: Paired t test; p value : considered Screening Period as the baseline; p-value <0.05 is significant.

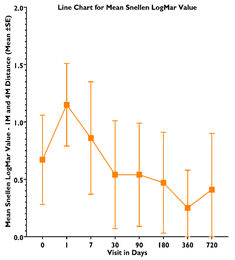


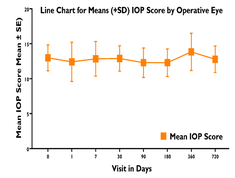
Figure 5: Line chart for Mean (±SD) LogMar BVCA in the Operated Eye

Continued improvement was observed by Day 30, where the mean dropped to 0.54, though this change did not reach statistical significance (p = 0.0803). On Day 90, the LogMar score remained at 0.54, with a significant improvement from baseline (p = 0.0016), suggesting steady visual recovery. This progress was maintained through Day 180 (mean = 0.47), although the change was not statistically significant (p = 0.0904). By Day 360, a substantial improvement was evident, with the mean decreasing to 0.25 and the change from baseline being highly significant (p = 0.0002). At Day 720, the mean LogMar value was 0.41, and the change from baseline remained statistically significant (p = 0.0438).

Statistics	Baseline	Day 1	Day 7	Day 30	Day 90	Day 180	Day 360	Day 720
Sample size (n)	20	17	19	19	17	17	19	18
Mean ± SD	12.98 ± 1.85	12.41 ± 2.81	12.84 ± 2.48	12.89 ± 1.82	12.29 ± 2.11	12.29 ± 1.96	13.84 ± 2.67	12.78 ± 1.90
Median	13	12	12	12	12	12	15	13
(Q1, Q3)	12, 14.5	10, 14	10, 15	11, 15	11, 13	12, 14	12, 16	12, 14
Min, Max	9, 16	8, 19	9, 17	11, 16	7, 17	7, 15	8, 17	10, 16
p value		0.7121	0.7764	0.9523	0.4217	0.3067	0.1200	0.9581

Table 4: Overall Summary of IOP Score of Operative Eye:
SD: Standard Deviation,
Min: Minimum; Max: Maximum; Q1: First Quartile; Q3; Third Quartile; CI: Confidence Interval;
Change from baseline = Post values–Baseline values;
Statistical Method: Paired t test;
p value :
considered Screening Period as the baseline; p-value <0.05 is significant.

Figure 6: Across all timepoints, none of the p-values fell below the significance threshold of 0.05, suggesting that the procedure did not result in statistically significant changes in IOP over time. Variability remained within expected ranges, and no consistent trend of increase or decrease in IOP was evident



Vitals and Adverse Effect Screening

Statistics	Systolic Blood Pressure		Diastolic Blood Pressure		Temperature		Pulse rate		Weight	
	Screening	Unscheduled	Screening	Unscheduled	Screening	Unscheduled	Screening	Unscheduled	Screening	Unscheduled
Sample Size (n)	20	3	20	3	20	3	20	3	20	3
Mean ± SD	127.5 ± 17.04	130.0 ± 0.00	80.60 ± 8.66	80.00 ± 0.00	97.00 ± 0.00	97.00 ± 0.00	74.80 ± 6.03	74.00 ± 0.00	59.95 ±10.24	57.33 ± 7.51
Median	130.00	130.00	80.00	80.00	97.00	97.00	74.00	74.00	59.50	53.00
Min, Max	96.00, 166.00	130.00, 130.00	60.00, 100.00	80.00, 80.00	97.00, 97.00	97.00, 97.00	62.00, 90.00	74.00, 74.00	37.00, 88.00	53.00, 66.00
(Q1, Q3)	120.00, 140.00	130.0, 130.00	80.00, 80.00	80.00, 80.00	97.00, 97.00	97.00, 97.00	73.00, 76.00	74.00, 74.00	55.00, 65.00	53.00, 66.00

Table 5: Summary of Vitals. SD: Standard Deviation; Min: Minimum; Max: Maximum; Q1: First Quartile; Q3; Third Quartile; Statistical Software: R-software 4.4.3

Subject ID	Adverse Event	Adverse Event Start Date	Adverse Event Stop Date	Adverse Event concomitant medication	Adverse Event Severity	Action	Outcome
1	Delayed epithelialization	15/12/2022	19/12/2022	Yes	Mild	BCL Replaced addition 5 days	Resolved
2	Delayed Epithelialization	22/12/2022	26/12/2022	Yes	Mild	BCL replaced additional 5days	Resolved
3	Delayed Epithelialization	22/12/2022	26/12/2022	Yes	Mild	BCL replaced additional 5 days	Resolved

Table 6: Summary of Adverse Events

Conclusion

- The use of alginate-preserved limbal stem and stromal cells has demonstrated safety and efficacy in the treatment of superficial corneal pathologies
- Our data indicate a clear pattern of gradual and sustained improvement in visual acuity post-treatment.
- The changes, particularly from Day 90 onwards, indicate a positive and sustained treatment effect, supported by statistical evidence and reduced variability in patient responses.
- The changes are both statistically and clinically meaningful.
- Overall, the treatment shows strong efficacy in improving vision in the operative eye.
- Further investigation is warranted to evaluate their therapeutic potential in larger cohorts of patients with similar conditions

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Competing interest & Financial disclosures: None **IEC Ref No: LEC 09-19-318** **ICSCR Reference No: 05-21-017**

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