88300_Auto_Edited.docx

WORD COUNT	3998	TIME SUBMITTED	16-OCT-2023 10:12AM
		PAPER ID	103493997

Name of Journal: *World Journal of Transplantation*

Manuscript NO: 88300

Manuscript Type: MINIREVIEWS

Focus on Limbal Stem Cell Deficiency and Limbal Cell Transplantation

Limbal stem transplantation

Emanuele Tonti, Gregorio Antonio Manco, Leopodo Spadea, Marco Zeppieri

Abstract

Limbal stem cell deficiency (LSCD) causes severe vision impairment and can lead to blindness, representing one of the most challenging ocular surface disorders. Stem cell deficiency can be congenital or, more often, acquired. The categorization of ocular surface transplantation techniques is crucial to achieve treatment homogeneity and quality of care, according to anatomic source of the tissue being transplanted, genetic source, autologous or allogenic transplantation (to reflect histocompatibility in the latter group), cell culture and engineered tissue techniques. The aim of this minireview is to provide a summary about the management of limbal stem cell deficiency, from clinical characteristics and therapeutic outcomes to the development of novel therapeutic approaches. The manuscript also briefly summarizes recent findings in current literature and outlines the future challenges to overcome in the management of the major types of ocular surface failure.

Key Words: limbal stem cell deficiency (LSCD); conjunctival limbal autograft (CLAU); conjunctival limbal allograft (CLAL); keratolimbal allograft (KLAL); cultivated limbal epithelial transplantation (CLET); simple limbal epithelial transplantation (SLET)

Tonti E, Manco GA, Spadea L, Zeppieri M. Focus on Limbal Stem Cell Deficiency and Limbal Cell Transplantation . *World J Transplant* 2023; In press

Core Tip: Limbal cell transplantation has been developed for the management of LSCD, to improve this condition and related complications, ameliorating visual acuity and quality of life of affected patients. Some of the limitation include the lack of specific markers and standardized methods to identify limbal stem cells, as well as the need to standardize the choice of therapeutic options which have diversified over the years and have evolved in terms of technology, efficacy, and safety. This clinical update review is to enable clinicians with the best evidence and current recommendations for managing their patients within the most advanced limbal cell transplant techniques.

INTRODUCTION

The primary function of cornea is to refract light, its function directly depends on its transparency. One of the factors that is implied in cornea's transparency is epithelium integrity. The corneal epithelium is a non-keratinized multilayer cuboid epithelium that covers the cornea starting from the limbus, where the junction between conjunctiva and cornea is. It is capable of self-renewing thanks to the presence of staminal cells. Corneal epithelial limbal stem cells (LSCs) reside preferentially in the basal layer of peripheral cornea in the limbal zone[1, 2]. There are no current specific marker for these cells. The research methods used for the identification of these cells tend to be indirect. The presence of stem cell-associated markers such as p63 and the absence of differentiated cell-markers such as CK12 (for corneal epithelium) or CK19 (for conjunctiva) indicate the putative stem cells[3, 4].

With regards to the predominant theory about corneal epithelium regeneration, LSCs asymmetrically divide in transient amplifying cells (TAC) that migrate centripetally and anteriorly and differentiate in squamous cells [5]. Several current studies suggest that, in experimental models, some staminal cells could reside outside the limbus[5-7].

The presence of LSCs is crucial to inhibit the proliferation of the conjunctival epithelium on corneal surface, the reduction of their number leads to conjunctivalisation of the corneal surface, persistent and recurrent epithelial defects, scarring and ulceration of the cornea. This condition is called limbal stem cell deficiency (LSCD). LSCD can be primary or secondary. Primary causes can occur for genetic pathologies or idiopathic, while the acquired can occur for traumas or autoimmune pathologies (Table 1) [8]. LSCD presents nonspecific symptoms such as discomfort, pain, photophobia, and decreased vision in more severe cases. The signs of LSCD depend upon pathology severity, starting from focal areas with stippled staining pattern, loss of clarity, epithelium hyperreflectivity on AS-OCT and flattening of Vogt palisades. In severe cases, it is possible to have conjunctivalisation of the cornea, whorl pattern in fluorescein staining, superficial corneal neovascularization, persistent epithelium defect (PED), stromal scarring or sterile melts[9].

These signs can affect just some portion of the cornea with a clear demarcation between normal and abnormal areas, accordingly with the extension LSC damage. In the cases of traumatic etiologies, LSCD is commonly asymmetrical. In autoimmune and congenital etiologies, LSC damage is commonly symmetrical. LSCD diagnosis is clinical in frank cases, but it can be confirmed by diagnostic investigation in subtle situations. There are several reliable tests in the diagnosis of LSCD. Understanding the underlying cause of LSC damage and starting the adequate therapy is fundamental to ensure good outcomes of LSCD treatments.

The aim of this minireview is briefly summarize the important issues regarding the clinical characteristics and management of patients with limbal stem cell deficiency, in addition to summarizing the therapeutic outcomes to the development of novel therapeutic approaches, future challenges, and recent findings in current literature.

CONCLUSION

INTRODUCTION

The primary function of cornea is to refract light, its function directly depends on its transparency. One of the factors that is implied in cornea's transparency is epithelium integrity. The corneal epithelium is a non-keratinized multilayer cuboid epithelium that covers the cornea starting from the limbus, where the junction between conjunctiva and cornea is. It is capable of self-renewing thanks to the presence of staminal cells. Corneal epithelial limbal stem cells (LSCs) reside preferentially in the basal layer of peripheral cornea in the limbal zone[1, 2]. There are no current specific marker for these cells. The research methods used for the identification of these cells tend to be indirect. The presence of stem cell-associated markers such as p63 and the absence of differentiated cell-markers such as CK12 (for corneal epithelium) or CK19 (for conjunctiva) indicate the putative stem cells[3, 4].

With regards to the predominant theory about corneal epithelium regeneration, LSCs asymmetrically divide in transient amplifying cells (TAC) that migrate centripetally and anteriorly and differentiate in squamous cells [5]. Several current studies suggest that, in experimental models, some staminal cells could reside outside the limbus[5-7].

The presence of LSCs is crucial to inhibit the proliferation of the conjunctival epithelium on corneal surface, the reduction of their number leads to conjunctivalisation of the corneal surface, persistent and recurrent epithelial defects, scarring and ulceration of the cornea. This condition is called limbal stem cell deficiency (LSCD). LSCD can be primary or secondary. Primary causes can occur for genetic pathologies or idiopathic, while the acquired can occur for traumas or autoimmune pathologies (Table 1) [8]. LSCD presents nonspecific symptoms such as discomfort, pain, photophobia, and decreased vision in more severe cases. The signs of LSCD depend upon pathology severity, starting from focal areas with stippled staining pattern, loss of clarity, epithelium

hyperreflectivity on AS-OCT and flattening of Vogt palisades. In severe cases, it is possible to have conjunctivalisation of the cornea, whorl pattern in fluorescein staining, superficial corneal neovascularization, persistent epithelium defect (PED), stromal scarring or sterile melts[9].

These signs can affect just some portion of the cornea with a clear demarcation between normal and abnormal areas, accordingly with the extension LSC damage. In the cases of traumatic etiologies, LSCD is commonly asymmetrical. In autoimmune and congenital etiologies, LSC damage is commonly symmetrical. LSCD diagnosis is clinical in frank cases, but it can be confirmed by diagnostic investigation in subtle situations. There are several reliable tests in the diagnosis of LSCD. Understanding the underlying cause of LSC damage and starting the adequate therapy is fundamental to ensure good outcomes of LSCD treatments.

The aim of this minireview is briefly summarize the important issues regarding the clinical characteristics and management of patients with limbal stem cell deficiency, in addition to summarizing the therapeutic outcomes to the development of novel therapeutic approaches, future challenges, and recent findings in current literature.

METHODS

We conducted a search of the literature published between January 1, 2002 to December 1, 2022 using MEDLINE (PubMed). The database was first searched using the key words “Limbal Stem Cell Deficiency, Limbal Cell Transplantation, Limbal Stem Cell Deficiency AND Limbal Cell Transplantation, LSCD AND CLAU, LSCD AND CLAL, LSCD AND CLET, LSCD AND SLET”. We considered only studies in English and those referring to humans and with an abstract, thus reducing the count to 301 papers. The

reference lists of all retrieved articles were assessed to identify additional relevant studies. The research of articles was performed using PubMed (https://pubmed.ncbi.nlm.nih.gov) and Reference Citation Analysis (https://www.referencecitationanalysis.com) Only articles with an abstract were considered. A quality score was calculated for each article using a check list. Each study was independently assessed by at least two reviewers (Tonti E and Zeppieri M), and rating decisions were based on the consensus of the reviewing authors. The most common surgical techniques highlighted in the most relevant studies are shown in Table 1.

DIAGNOSIS AND STAGING OF LSCD

Impression Cytology

A filter paper of nitrocellulose or acetate cellulose is applied over the cornea or the conjunctiva to obtain cells from the ocular surface. Repeating the sampling on the same area allow to obtain cells from the deeper layers, this makes the sampling more reliable. The specimens are processed with various stains searching for goblets cells[9-11]. The presence of these cells indicates the invasion of conjunctival epithelium over the cornea, but their absence does not exclude LSCD, in fact, in some cases, as Stevens-Johnson syndrome or chronic inflammatory diseases, the number of conjunctival goblet cells can be markedly reduced, and their identification can be difficult[11]. Differentiate corneal epithelium cells from conjunctival epithelium cells, instead, is possible only by immunohistochemistry. As mentioned before, CK2 and 12 are specific for mature corneal epithelium, CK3 for conjunctiva and corneal epithelium, CK7, 13 and 19 are specific for conjunctival epithelium. Another used marker is mucin 5AC (MUC5AC), but it has a low sensitivity. Impression cytology is also useful to analyze the result of the LSCD therapies[12].

In-Vivo Confocal Microscopy (IVCM)

With this exam it is possible to acquire pictures of the corneal microstructures without collecting specimens. The presence of goblets cells in a corneal IVCM, as seen in impression cytology, confirms the diagnosis of LSCD, but their absence can't exclude the diagnosis because in this exam is scanned just a small area and the morphology of goblet cells can be difficult to recognize in IVCM[13]. In LSCD the density of the basal cells of corneal epithelium is decreased and the mean size of cells is increased, these findings correlate with the severity of the pathology[14]. Other findings are intraepithelial cystic lesions surrounded by goblet cells and the decrease of the density of the sub basal nervous plexus [15].

Anterior Segment Optical Coherence Tomography (AS-OCT)

This is a non-invasive and low operator dependence imaging tool. LSCD has been associated to epithelial thinning at cornea and limbus, but these signs are not specific for LSCD[16]. With volumetric scans it is possible to study the status of Vogt palisades, their thinning (or absence) is associated to areas with thinned epithelium. The analysis of the reflectivity of epithelium and stroma in LSCD show that epithelial reflectivity vary more than stromal, and the ratio between them could be a diagnostic tool for LSCD, furthermore this ratio tends to return to normal values after LSC transplantation, even if do not return to normal values[17, 11].

LSCD staging is based on the clinical presentation, 5 mm central cornea affection and limbal involvement are the two parameters evaluated (Table 2). The most important factor is corneal involvement, in the first stage central 5 mm are not involved, in the second are partially affected and in the final stage the entire corneal surface is involved. Every stage is divided in A, when limbus involvement is less than 50%, B, if it's more that 50% but non-complete, and C if limbus is completely affected. Correct staging is a useful tool for therapeutic decisions, but it is also important to evaluate palpebral and adnexa status and to control the underlying pathology, if the LSCD is secondary to other pathologies[8]. Figures 1-3 show different stages of stem cell deficiency.

Figure 1: Limbal stem cell deficiency stage III, in which the entire corneal surface is involved.

Figure 2: Partial stem cell deficiency (stage II-A).

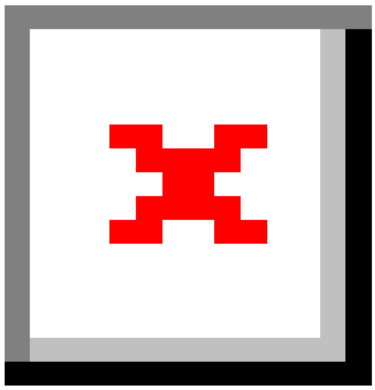

Figure 3: Post traumatic limbal stem cell deficiency with central cornel scarring.

OCULAR SURFACE STEM CELL TRANSPLANTATION: CLINICAL OUTCOMES

Over the past two decades, a variety of ocular surface rehabilitation treatments have been developed. The ocular surface is rehabilitated by improving the ocular surface environment, ensuring control inflammation, good lubrication, lids closure, eliminating keratinization and symblepharon. A favorable environment is crucial for restoring the normal corneal phenotype and proper corneal clarity[18]. Cornea transplantation can be considered for corneal clarity restoration in patients with LSCD, but results and visual outcomes tend to be limiting over time because of the inability of the LSC to regenerate and maintain the transparency of the epithelium[31].

A number of transplantation procedures have been used over the past years, and many of them have been labeled using different terminology. These procedures include

autologous and allograft conjunctival transplantation[19-21], keratoepithelioplasty[22], homotransplantation of limbal cells[23], limbal transplantation[24], homotransplantation of limbal cells[25], autologous and allograft limbal transplantation (Table 3)[26-29].

- *Conjunctival Limbal Autograft (CLAU)*

CLAU, first described by Tseng and Kenyon in 1989, is one of the most used techniques for limbal stem cells transplantation in unilateral LSCD, it consists in taking a portion of limbal conjunctiva (usually from 3 to 6 clock hours) from the fellow eye and implant it in the affected eye, with or without amniotic membrane (AM) transplantation. CLAU has been successfully used to treat different pathologies and LSCD of different etiologies and different severities, even in cases of total corneal involvement[32]. The donor eye should be exanimated with particularly attention to exclude any sign of LSCD to avoid a iatrogenic LSCD, even if this represents a remote possibility[33,34,39,46].

The inflammatory status of the graft can reduce the transplantation success rate, so topical steroids could be useful before the graft harvesting[36]. There is not a universal accepted consensus about the size of limbal grafts, generally harvested at 12 and 6 clock hours, where Vogt palisades are more developed, the first techniques used wide graft (8 clock hours), but successfully results has been achieved with smaller graft and new promising techniques uses two grafts each of about one clock hour combined with Amniotic Membrane Transplantation (AMT)[35-38].

Amniotic membrane could be transplanted even in the donor eye when large grafts are taken to reduce the risk of LSCD due to its capacity of facilitating the in-vivo expansion of LSCs[41,42]. Generally, the recipient bed is prepared doing a peritomy 4-5 mm from the limbus and dissecting the corneal pannus, the dissection of corneal layers should be avoided, stromal opacities are better treated afterwards with keratoplasty[40]. The limbal epithelial graft are fixed around the cornea and the posterior margin is sutured to the conjunctiva. Between the graft and the ocular surface could be interposed a layer of AM

that seems to increase the rates of success and the rapidity of the healing process, especially with little grafts, but a study with wide grafts showed no significant differences between the AMT group and the other without[42-44].

The great advantage of autologous transplantation is the immunosuppressive therapy spearing, so, generally, the only medication needed are topical antibiotics, topical corticosteroids, and the application of a scleral lens to protect the graft from the mechanical stress of winking, sometimes is indicated a temporary tarsorrhaphy[45]. A meta-analysis of 2020 found that the overall success rate of CLAU is of 83.2%; 95%CI, defining "success" the reconstruction of an intact epithelium and a stable ocular surface. These data involve 16 articles, 505 eyes, most of them with chemical or thermal injury[46].

- *Living-Related Conjunctival–Limbal Allograft (lr-CLAL) Transplantation*

In this technique, surgical procedure is the same of CLAU, but the graft is harvested from a living related, this makes it suitable even for severe bilateral LSCD. To decrease the chances of rejection, the donor must be the best human leukocyte antigen (HLA) matched available relative, generally a parent or a sibling. In 100% HLA compatibility cases, immunosuppressive therapy is not needed, in other cases is required administration of 6-12 mo of oral corticosteroids 10 mg/Kg/die and oral cyclosporin A 10 mg/Kg/die, subsequently tapered for maintenance dose during all the follow up period. Some protocols added azathioprine to previous drugs and other used tacrolimus and mycophenolate mofetil[47,48], other authors administered oral cyclosporin for more than 6 mo and topical cyclosporin continued indefinitely unless toxic effects onset[49]. Patients under immunosuppressive therapy must be checked often for liver and kidney function.

- *Keratolimbal Allograft (KLAL)*

KLAL is an allogenic transplantation from a cadaveric donor. The graft is prepared dissecting and removing the limbus and a peripheral portion of the cornea of the donor eye, then the stromal portion is dissected carefully to preserve the conjunctival and limbal epithelium. The graft is then sutured to the peripheral cornea and a patch of AM is generally transplanted to ensure better outcomes[50-52]. Tissue from the youngest possible donor with an upper limit of 50 years is recommended. Surgery should be performed within 72 h as the cells are expected to be more active and vital[53-55]. The recipient bed is prepared the same way of CLAU and lr-CLAL. HLA matching is recommended, and an immunosuppressive therapy, similar to lr-CLAL, is generally needed.

Holland *et al* developed the Cincinnati procedure Combining lr-CLAL and KLAL. In this technique, two portion of healthy limbus conjunctiva are harvested by a HLA matched living donor, and the corneoscleral rim is taken from a cadaveric donor. The conjunctival tissue is placed at 12 and 6 o'clock in the same anatomical orientation, the corneoscleral tissue is placed at 3 and 9 o'clock. With this procedure ocular surface stability was achieved in 54,2% and an improvement was achieved in 33.3%, and 75% had an improvement of the visual acuity[67]. The same authors described even the modified Cincinnati procedure that combines CLAU to KLAL, achieving ocular surface stability in 82% of patients and ocular surface improvement in 18%[68]. Both techniques require an immunosuppressive therapy like KLAL one[67-68].

- *Tissue Engineering for Reconstruction of the Corneal Epithelium*

In this group of techniques, a small portion of epithelium is taken from a donor, cultivated to expand its surface, and then transplanted. The advantage of these techniques is that with a limited amount of harvested tissue it is possible to generate a considerable amount of epithelium to transplant, so even in severe bilateral LSCD it is possible an autologous transplantation, but the cultivation process needs an advanced laboratory and a relevant amount of resources, so just few centers actually perform

these kind of surgeries. The harvested corneal tissue can belong to a living donor (the patient itself, a living relative, or a living nonrelative person) or from a cadaveric donor, but some techniques use other epithelia such as the oral one (ex vivo oral mucosa autograft, EVOMAU, also called cultivated oral mucosa epithelial transplantation, COMET).

Furthermore, most of them are still experimental procedure non suitable for routinary application except for Holoclar (ex vivo expanded autologous human corneal epithelial cells containing stem cells), first described in 1997 by Pellegrini *et al*, that achieved EMA authorization for commercial purposes in 2015[56].

Cultured Limbal Epithelial Stem Cells for Reconstruction of the Corneal Epithelium:
Holoclar is a cultured limbal epithelial transplantation (CLET) procedure that starts with the enzymatical dissociation of the sample and the seeding of the cells in a layer of irradiated mouse feeder cells with grow factors ad antibiotics. After this step, cells are cryopreserved and samples are tested, some of them are stored in case of failure of the first graft. Primary cultures are then seeded into an antibiotic-free fibrin matrix discs and cultivated again. Epithelized discs are then shipped to the clinic, shaped by the surgeon, and implanted like in CLAU technique. There is not a standard procedure for CLET, in fact, cultivation procedure in literature varies for substrate.

Most of them uses amniotic membrane, and cultivating milieu, but the overall success rate of this technique is 71.8%, when cultivated cells are autologous the ocular surface stability is maintained for long follow up periods[39, 58-66]. Most of patients are typically affected by LSCD for chemical injury but this technology it is used also to treat LSCD due to autoimmune pathologies and congenital pathologies.

Another technique is autologous conjunctival epithelial cells cultivated *ex vivo* (EVCAU), in which the cultivated tissue is forniceal conjunctiva. The specimen is placed

on a denuded human amniotic membrane and submerged in a culture medium with grow factors and antibiotics. The cultivated tissue is then shaped and transplanted to the prepared corneal surface. A study with 12 eyes reported a success rate of 66.6% and 16.6% of partial success (conjunctival epithelial ingrowth recurred in 2 corneal quadrants), but we have no other data about the clinical outcomes in humans[57].

- *Simple Limbal Epithelial Transplantation (SLET)*

SLET is a recent procedure for unilateral disease, seeds donor stem cells directly on an amniotic membrane placed on the recipient's ocular surface, completely obviating any need for laboratory conditions of expansion[69]. Although CLET reduced the complications of CLAU, cell expansion required a clinical-grade lab with regulatory approvals, which was and continues to be very expensive to build and maintain. Simple limbal epithelial transplantation (SLET), introduced by Sangwan *et al* in 2012, combined the benefits of CLAU and CLET while avoiding the limitations of both strategies[69].

Unilateral LSCD is the primary indication for autologous SLET. Ocular burns are the most common cause of unilateral LSCD, so it is not surprising that this indication is covered by almost all of the published literature on autologous SLET. More recently, the first case reports of allogenic SLET in cases of bilateral LSCD have been proposed and involved patients with severe chemical burns and dry eyes, respectively[70,71]. In SLET technique, in the superiorlimbal district of the unaffected contralateral eye, a portion of 2 x 2 mm of limbal tissue is removed under topical anesthesia and placed in balanced saline solution.

The corneal surface is exposed by removing the fibrovascular corneal pannus after 360-degree conjunctival peritomy and peribulbar anesthesia is induced.

Epithelial side up, human amniotic membrane (AM) is grafted over the cornea, secured with fibrin glue, and the margins are trimmed to fit the external conjunctival borders. Eight to ten tiny pieces of the limbal sclerocorneal tissue are cut into pieces and adhered

to the AM in a circular pattern using fibrin glue, sparing the optical zone[69]. In a study involving six patients with total unilateral LSCD, visual acuity improved in four of the recipient eyes (66.6%), going from 20/200 or worse before SLET surgery to 20/60 or better afterward. None of the donor eyes experienced any complications. It took 9.2 mo on average to follow up[69].

FUTURE PERSPECTIVES AND CONCLUSIONS

Patients with severe ocular surface disease need to be treated in a methodical, step-by-step manner. To achieve the best results in the rehabilitation of the ocular surface, it is crucial to select the patient's most appropriate strategy of treatment. The underlying pathology, the extent and severity of ocular surface disease, including the degree of stem cell damage, unilaterality or bilateralism of the condition, the presence or absence of conjunctival inflammation, whether tear production is normal (significantly altered, or absent) the patient's age, and systemic co-morbidities are important factors in the choice of regimen among the various surgical procedures proposed for the treatment of LSCD.

The development of xenobiotic-free culture systems and the standardization of culture conditions are two improvements that must be made in order to advance the therapeutic approach. Additionally, in order to guarantee the functionality and long-term regeneration of the transplants, tissue engineering strategies must incorporate a kind of quality control, verifying the preservation of stem cells during the culture process.

Clarifying the signaling pathways that control stem cell function and fate *in vivo* and *in vitro* is one of the remaining challenges. Future trends include the creation of biomimetic scaffolds that can deliver drugs, growth factors, or signaling molecules to

help further promote cell function and tissue regeneration in addition to acting as structural supports for living cells.

88300_Auto_Edited.docx

ORIGINALITY REPORT

7%
SIMILARITY INDEX

PRIMARY SOURCES

1	Schlötzer-Schrehardt, Ursula, Naresh Polisetti, Johannes Menzel-Severing, and Friedrich E. Kruse. "Tissue Engineering for Reconstruction of the Corneal Epithelium", Ocular Surface Disease Cornea Conjunctiva and Tear Film, 2013. Crossref	44 words — 1%
2	entokey.com Internet	43 words — 1%
3	Sheraz M Daya, Clara C Chan, Edward J Holland. "Cornea Society Nomenclature for Ocular Surface Rehabilitative Procedures", Cornea, 2011 Crossref	40 words — 1%
4	www.researchgate.net Internet	34 words — 1%
5	www.ijo.in Internet	32 words — 1%
6	Zaman shah, Ibrar Hussain, Khalid Mehmood, inayat ullah, Nadeem Butt, Pir Salim Mahar. "Clinical efficacy of autologous simple Limbal epithelial transplantation for stem cell deficiency in ocular surface burn", Research Square Platform LLC, 2023 Crossref Posted Content	28 words — 1%

7	Lakshmi Nagendra, Cornelius James Fernandez, Joseph M Pappachan. "Simultaneous pancreas-kidney transplantation for end-stage renal failure in type 1 diabetes mellitus: Current perspectives", World Journal of Transplantation, 2023 Crossref	20 words — < 1%
8	downloads.hindawi.com Internet	20 words — < 1%
9	coek.info Internet	14 words — < 1%
10	"Corneal Regeneration", Springer Nature, 2019 Crossref	13 words — < 1%
11	f6publishing.blob.core.windows.net Internet	12 words — < 1%

EXCLUDE QUOTES	ON	EXCLUDE SOURCES	< 12 WORDS
EXCLUDE BIBLIOGRAPHY	ON	EXCLUDE MATCHES	< 12 WORDS