History, presence, and future of mitomycin C in glaucoma filtration surgery

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History, presence, and future of mitomycin C in glaucoma filtration surgery

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Purpose of review

Mitomycin C (MMC) is an alkylating agent with extraordinary ability to crosslink DNA, preventing DNA synthesis. By this virtue, MMC is an important antitumor drug. In addition, MMC has become the gold standard medication for glaucoma filtration surgery (GFS). This eye surgery creates a passage for drainage of aqueous humor (AqH) out of the eye into the sub-Tenon's space with the aim of lowering the intraocular pressure. A major cause of failure of this operation is fibrosis and scarring in the sub-Tenon's space, which will restrict AqH outflow. Intraoperative application of MMC during GFS has increased GFS success rate, presumably mainly by reducing fibrosis after GFS. However, still 10% of glaucoma surgeries fail within the first year.

Recent findings

In this review, we evaluate risks and benefits of MMC as an adjuvant for GFS. In addition, we discuss possible improvements of its use by adjusting dose and method of administration.

Summary

One way of improving GFS outcome is to prolong MMC delivery by using a drug delivery system.

Keywords

Drug delivery system (DDS), fibrosis, GFS failure, glaucoma filtration surgery (GFS), mitomycin C (MMC)

INTRODUCTION

Glaucoma is the leading cause of irreversible blindness worldwide [1,2,3]. By 2020, the prevalence of glaucoma is estimated to be around 80 million people worldwide [4]. High intraocular pressure (IOP) is one of the most important risk factors of glaucoma [5–7]. All current glaucoma treatment modalities aim at lowering IOP, initially with eye drops and/or laser treatment [1,8]. If these treatments do not have the desired effect, a surgical intervention can be performed, the so-called glaucoma filtration surgery (GFS) [1,8–10]. GFS is the last resort treatment option for glaucoma patients [7,11]. Current standard GFS techniques (e.g. trabeculectomy or long-tube glaucoma implant/shunt) have been practiced for several decades [3,6] and are the most effective treatments to achieve sustained IOP reduction [9]. During GFS, an artificial passage (e.g. by placing a shunt) [2,9,12] is created for drainage of aqueous humor (AqH) from the anterior chamber into the space under the conjunctiva and Tenon's capsule, forming a filtering bleb. This procedure will increase outflow of AqH and reduce

IOP. GFS requires a substantial follow-up care and does not always lead to a permanent IOP reduction [13]. An important complication is the development of fibrosis (scarring) of the filtering bleb [4,14,15,16], which often reduces the outflow of AqH through the operatively created drainage

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KEY POINTS

- In order to further reduce the GFS failure rate, we roughly have two options, either improve MMC application or develop new drugs.
- We suggest that prolonged presence of MMC may control fibrosis over a longer period of time, because repeated injections of MMC have shown positive results.
- Eventually, new, less toxic GFS drugs will be developed to further increase GFS success rate.

passage; as a consequence, IOP will rise again [9]. This phenomenon of IOP rise after GFS is classified as a GFS-failure and most patients need to restart their medication and/or undergo a revision or new surgery procedure.

The introduction of mitomycin C (MMC) administration to augment the outcome of trabeculectomy in the early 1990s resulted in improved long-term IOP control [17], presumably by its anti-fibrotic action [12]. Currently, intraoperative MMC administration in the sub-Tenon's space with a sponge soaked in MMC is the gold standard anti-fibrotic treatment in GFS [8,9]. Additionally, the use and application of MMC in ophthalmology, especially in glaucoma procedures, has been increasing in recent years, because of its modulatory effects on wound healing [18]. Unfortunately, MMC administration does not completely prevent failures of current GFS procedures. In order to reduce the number of failures, new so-called minimally invasive glaucoma surgical (MIGS) devices and procedures have been developed and have been made available for several years [19²²,20,21]. These MIGS devices are less stressful for patients [19²²,20,21], but unfortunately also not always successful [22]. There are currently three general types of MIGS devices (implants) that shunt from the anterior chamber to, (1) Schlemm's Canal (iStent, Hydrus), (2) the supraciliary space (new devices in development), and (3) to a bleb formed under the conjunctiva and Tenon's capsule, (Allergan XEN-gel stent and the Santen PRESERFLO^{TM} MicroShunt) which emulates GFS [21]. Importantly, MMC administration still remains necessary in GFS-based subconjunctival-based MIGS for suppressing the fibrotic reaction. Recent results show that a substantial proportion of MIGS placed in the sub-Tenon's space, augmented with MMC, still show an increase in IOP within the first year, too often leading to failure [23]. Overall, we conclude that despite the development of new GFS methods the administration of MMC is still required. A more important concern is that failure

rates of the new MIGS methods with onetime intra-operative MMC administration remain relatively high.

The aim of this literature review is to evaluate the risks and benefits of MMC as an adjuvant for GFS. In addition, we discuss possible improvements of MMC use. The question is whether MMC is here to stay, is it time to adjust its way of application or is it time to find a new drug and medication regimen?

MITOMYCIN C AND ITS USE IN GLAUCOMA FILTRATION SURGERY

Physical and chemical properties

MMC was isolated from the broth of the *Streptomyces caespitosus* in Japan in 1954 [8,17,18,24,25,26,27]. MMC is isolated as blue violet crystals [26]. The molecular weight of MMC (C₁₅H₁₈N₄O₅) is 334.33 Daltons. MMC is readily soluble in water (0.5 mg/ml) and organic solvents [26,28³⁰]. The melting point is 360°C [29]. MMC solutions, in 0.9% saline, are stable for at least 6 h at 37°C [30].

Reactive properties

MMC is an alkylation agent targeting DNA, RNA, proteins, and lipids. Interestingly, MMC was postulated to have two alkylating centers, the 1,2-aziridine (C1 position) and 10-carbamate group (C10 position) (Fig. 1) [27]. As an example, the chemical reaction (reductive alkylation) with DNA is illustrated in Fig. 2, adapted from Verweij and Pinedo [26].

FIGURE 1. Chemical structure of MMC. (A) 1,2 aziridine with the first alkylating reactivity at the C1 position (see red dot), (B) 10-carbamate group with a second alkylating center at the C10 position (see red dot).

FIGURE 2. Reductive activation and DNA alkylation of MMC, adapted from Verweij and Pinedo [26]. (A) MMC, (B) intermediate of MMC, (C) unstable reactive intermediate of MMC, (D) quinone methide (has a high alkylating reactivity at the C1 position), (E) MMC-DNA adduct (with a second alkylating center at C10), (F) MMC cross-linked DNA adduct. MMC, mitomycin C.

The interaction of DNA with MMC results in the alkylation of multiple DNA guanine residues [31]. The clinically used antitumor agent MMC alkylates DNA upon reductive activation and forms in this process six covalent DNA adducts [27]. First, a one- or two-electron reduction (Fig. 2A and B) occurs under anaerobic conditions, followed by a spontaneous loss of methanol that leads to the formation of an unstable reactive intermediate (Fig. 2B and C).

Thereafter, rearrangement of the reactive intermediate (Fig. 2C) will be followed by a nucleophilic addition of DNA that results in a mono-alkylated product (Fig. 2D). Intramolecular displacement of the carbamate group would then result in the cross-linked adduct (Fig. 2E and F).

Overall, DNA alkylation by MMC will block DNA synthesis and consequently inhibit cell proliferation [8,31]. In addition, several of the formed

DNA adducts are capable of inducing cell death in cancer cells since they can alkylate and cross-link DNA (for review, see [27]).

Activation of MMC within the cell is influenced by several factors. First, it is known that MMC is activated by enzymatic reduction within the cell, a process mediated by cytochrome P-450 reductase and that occurs most effectively in a hypoxic environment [24,26]. Second, it has been noted that MMC activation is also nicotinamide adenine dinucleotide phosphate (NADPH)-dependent [29]. Third, it is known that MMC activates the PI3K/Akt signaling pathway in a p53-dependant manner [27,32]. Activation of MMC will result in alkylation/crosslinking of DNA as shown in Fig. 2.

In addition, it has been shown that MMC crosslinks the complementary strands of the DNA double helix especially in CpG-sites [25,26]. Furthermore, activated MMC is most effective in the late G1- and S-phases of the cell cycle [17,18,26], but is not essentially cell cycle specific [17]. Because MMC is relatively nonspecific and reacts with cells in every phase in the cell cycle, it causes cytotoxicity by lipid peroxidation and by damage to DNA and protein [33].

MMC is not only known as an alkylating agent [24,32], but also as an antibiotic [34], cross-linking reagent [35], and a nucleic acid synthesis inhibitor [36]. In addition to the specific inhibition of DNA synthesis due to alkylation, inhibition of RNA and protein synthesis seem to be nonspecific manifestations of MMC cell toxicity [26,29]. The use of MMC as an anticancer agent or as an antifibrotic/antiadhesion agent relies on exactly the same molecular mechanism of action, namely the formation of covalent adducts of MMC with DNA [31].

Common applications of MMC

By virtue of the actions as described above, MMC is used as an antitumor, antibiotic, antiproliferative, antifibrotic, and immunosuppressive agent. The antitumor MMC has shown clinical activity in a number of cancers, including stomach cancer, breast cancer, and cervical cancer [26,37,38]. Furthermore, MMC is known to have immunosuppressive properties and has been shown *in vitro* to inhibit B-cell, T-cell, and macrophage proliferation and impair antigen presentation, as well as the secretion of interferon gamma (IFN- γ), tumor necrosis factor alpha (TNF- α), and interleukin (IL)-2 [36]. Despite these properties it is not often used as an immunosuppressive agent in the clinic. Its use in ophthalmology has been increasing in recent years, because of its modulatory effects on wound healing (antiproliferative, antifibrotic) [18]. In this application,

MMC leads to an almost complete inhibition of fibroblast proliferation [24] and therefore a significant reduction of fibrosis [39].

GFS and cause of failures

Increased IOP can be caused by a myriad of factors contributing to either overproduction of aqueous humor (AqH) by the ciliary body or increased resistance in the AqH outflow paths from the anterior chamber [2]. Conventional outflow from the anterior chamber is through the trabecular meshwork into Schlemm's Canal, subsequently to the collector channels and then into the venous plexus in the sclera [2]. The secondary outflow path is into the supraciliary space and then into the venous plexus in the sclera [2]. If any of these paths are of high resistance, the IOP will become high. GFS reduces IOP by increasing the outflow of AqH out of the anterior chamber through an artificial passage that bypasses the trabecular meshwork, Schlemm's Canal, the collector channels and the venous plexus (e.g. by trabeculectomy or by placing a tube shunt) [6,9,11]. AqH is directed into the space under the conjunctiva and Tenon's capsule, the sub-Tenon's space, forming a filtering bleb [6,9,11]. Outflow from the bleb can be through the conjunctiva into the tear film or into the episcleral veins, whichever is of lower resistance [40]. The success of GFS is threatened by fibrosis of the filtering bleb, which progressively limits AqH outflow from the anterior chamber into the filtering bleb. Therefore, during GFS, surgeons use a onetime MMC administration intraoperatively within the wound bed to inhibit the postoperative formation of fibrotic and scar tissue [16]. This usually works very well in the first postoperative period. Unfortunately, despite this one-time intraoperative MMC treatment during GFS, fibrosis (scarring) occurs postoperatively and results in 5–10% failure rate every year [23]. Fibrosis, although being a part of the normal wound healing, will have the negative effect of blocking the artificial passage (for example by closing the flap or distal tube end) postoperatively [9,15,16]. This will result in a rise in IOP, requiring restart of glaucoma medications, an intervention (e.g. laser suture lysis, needling) or reoperation.

Wound healing and scarring is a complex process that consists of a series of events, including hemostasis (phase 1), inflammation (phase 2), proliferation (phase 3), and remodeling (phase 4) after surgical tissue trauma [4,9,15,16]. In more detail, the surgery starts with inducing a tissue trauma with at least some release of blood cells and plasma proteins, or hemostasis (phase 1), which will trigger a localized inflammatory response (phase 2). During

the inflammation phase the innate immune system will be activated and inflammatory cytokines will be released. In the early inflammation phase, platelet activation provides a rich source of mediators [such as platelet-derived growth factor (PDGF), vascular endothelial growth factor alpha (VEGF-A), and transforming growth factor beta type 1 (TGF- β 1)]. These factors enhance the recruitment of macrophages that also release different growth factors, such as transforming growth factors alpha and beta (TGF- α , TGF- β), PDGF, and fibroblast growth factor (FGF). Thereafter, 1 to 3 days after surgery, the proliferation phase (phase 3) ensues with propagation and invading of fibroblasts (this is driven for example by PDGF). Herein, transdifferentiation of fibroblasts into myofibroblasts will be induced by the growth factor TGF- β . Finally, this will enhance the expression of extracellular matrix (ECM) proteins to strengthen the ECM (phase 4).

This normal, naturally occurring wound healing reaction can produce an adverse result in GFS when scar tissue is generated in the filtering bleb, more specifically if this scar tissue encloses the ostium and scleral flap after trabeculectomy, or the distal end of the tube implant in MIGS procedure, limiting AqH outflow and resorption.

Introduction of MMC in GFS and its regulation

To prevent fibrosis, MMC is applied in conjunction with GFS. In 1983, Chen *et al.* [41] were the first to describe the efficacy of MMC treatment in enhancing bleb survival following trabeculectomy in eyes with a high risk of failure [24,41]. The introduction of MMC treatment in trabeculectomy surgeries has improved the long-term IOP control through delayed wound healing resulting from inhibition of fibroblast proliferation [17]. Chen *et al.* [42] originally reported in 1990 that application of a MMC solution at a concentration of 0.2 to 0.4 mg/ml for 5 min is adequate, and poses little risk of severe complications such as ocular hypotony [24,42]. However, they also suggested that 0.2 mg/ml for 5 min may be the safest effective dose [42]. Later, in 1995, Hung *et al.* [43] investigated the use of MMC by a subconjunctival injection prior to trabeculectomy in patients with refractory glaucoma [24,43]. In 2004, a survey of British ophthalmology consultants reported that 82% of the surgeons used antimetabolites [apart from MMC also 5-fluorouracil (5-FU) is used] during trabeculectomy [24,44]. Finally, MMC was reported as the most commonly used antimetabolite among American Glaucoma Society members in 2008 [24,45].

Legislation of MMC-usage is different in each country. Mitosol (mitomycin for solution, 0.2 mg/vial,

Kit for Ophthalmic Use) [46] and Mitozytrex [47] are the only FDA-approved ophthalmic formulations of MMC [24]. These are not allowed in Europe, where traditionally MMC is used off-label for ophthalmic purposes, and is freshly prepared by the hospital pharmacist prior to the scheduled surgery.

MMC administration during GFS and its results

During GFS, MMC solution is administered to the eye, at the location of the planned filtering bleb. It is known to be highly bioavailable for the target tissue [18,28⁹]. The MMC is mostly applied by inserting a sponge soaked in a concentration of 0.1 to 0.5 mg/ml MMC underneath a conjunctival/Tenon's flap onto the sclera, for 1 to 5 min [48]. For example, a sponge soaked in 0.2 mg/ml MMC for 2 to 3 min is the standard intraoperative treatment in for example many European sites (also at the University Eye Clinic Maastricht). Clinical studies have observed that most tissues become saturated with MMC after an intraoperative treatment of 1 min [24]. Furthermore, IOP levels in patients after GFS were similar between the conditions where MMC was used for 2 or 5 min [24]. In addition, sometimes MMC is also administered postoperatively by 0.1 ml subconjunctival injections of a 0.02 mg/ml solution (needling procedure) [48].

Xi *et al.* [49] reported that MMC is the most effective and popular anti proliferative drug for GFS, especially in eyes with high risk of failure. Intraoperative MMC treatment is effective in 90% of the GFSs whereby the created outflow pathway is open (meaning: MMC was able to inhibit the formation of fibrotic tissue after trabeculectomy and MIGS, which otherwise would close/block the outflow of AqH from the anterior chamber through the ostium (or MIGS shunt) into the filtering bleb) and the IOP remains in the normal-ranges (<15 mmHg for trabeculectomy).

Mechanistic aspects of MMC-treatment in GFS

MMC can function in GFS due to the alkylating effect of the drug [24,26,32]. Thereby, it inhibits cell proliferation/differentiation (e.g. fibroblasts into myofibroblasts; phase 3 of the wound healing process), kills cells (phase 1–3 of the wound healing process), and inhibits induction of inflammation (phase 2 of the wound healing process) [8,18,24,31,33,36,39]. A direct effect of MMC on the differentiation into M1-macrophages (pro-inflammatory; TNF- α) and M2-macrophages (anti-inflammatory; IL-10 and CD163) type macrophages has not been

studied yet. Most interestingly, MMC represents the most efficacious antifibrotic agent that is used as intraoperative treatment to attenuate the formation of fibrosis after trabeculectomy and to prolong the bleb survival [50]. Furthermore, fibroblasts of the Tenon's capsule are critical to the production of new extracellular matrix (such as collagen and glycosaminoglycans) in response to surgery [51,52]. Intraoperative MMC treatment inhibits the proliferation of these fibroblasts of Tenon's capsule [51,53,54].

In general, MMC inhibits processes in wound healing such as collagen synthesis, fibroblast migration and fibroblast proliferation (myofibroblast formation) [51,52,55]. Therefore, the artificial drainage passage will not be blocked by fibrotic tissue and AqH can freely flow out of the anterior chamber, which leads to an IOP reduction.

Failure of MMC-treatment

Overall, 10% of the GFSs fail within the first year and this is mostly caused by the formation of fibrotic tissue in/under the filtering bleb [56]. Clearly then, the current medication regimen is not fully effective in preventing fibrosis after GFS. Possible explanations of the failures could be: (1) MMC treatment is too short to prevent the formation of fibrotic tissue, (2) presence of pro-fibrotic agents in the AqH, (3) variation between patients, (4) toxic side effects of MMC treatment. Finally, fifth, it may be that MMC is not the optimal GFS medication after all.

MMC treatment is too short

The first reason for a GFS failure could be that the intraoperative MMC treatment is not sustained long enough to prevent fibrosis, considering for example that phase 3 of the wound healing process normally occurs after 5 to 7 days. There are several indications that the current onetime administration (within safe limits of concentration and duration) may not provide sustained levels of MMC. First, MMC has a relatively short half-life of plasma distribution (2–10 min) [26]. Second, MMC has a relatively short biological half-life (8–48 min) [36]. Third, MMC has a relatively rapid elimination time (25–90 min) [26,47]. Finally, the intraoperative MMC treatment and the corresponding rinsing techniques during GFS can result in quite variable local tissue levels of MMC; it may also vary considerably among surgeons [8]. In conclusion, MMC is rapidly cleared, has a fast half-life time, and the level of MMC present in the eye can differ between each GFS. All of these factors can contribute to why some patients fail after GFS and others do not. Of course, also variation between patients, will contribute to this.

Presence of pro-fibrotic agents in the AqH

Second, the tissue of the filtering bleb is continuously exposed to AqH drained from the anterior chamber. This contact normally does not occur, because AqH drains directly into Schlemm's canal and is carried away by the circulation. Multiple studies have shown that AqH of glaucoma patients contains a number of inflammatory cytokines, including IL-6 [57], IL-8 [57–59], TNF- α [60], serum amyloid A (SAA) [59], and the growth factor TGF- β 1 [59,61–65]. Expression changes of the ocular inflammatory cytokines (including TNF- α , IL-8, and SAA) and the growth factor TGF- β 1 in AqH, are known to play a role in IOP elevations in patients with primary open angle glaucoma [63]. These inflammatory cytokines and growth factors influence the wound healing response (namely, stimulating inflammation (phase 2) and proliferation (phase 3)), as described in paragraph about 'GFS and cause of failures'. TGF- β is known to induce the transdifferentiation of fibroblasts into myofibroblasts [4,9,15,16]. In conclusion, the continuous exposure of the filtering bleb to 'glaucomatous' AqH that contains increased inflammatory cytokines and TGF- β 1 will lead to a continuation of inflammation, proliferation, and finally fibrosis of the wound healing process and could therefore be one of the reasons why some patients will develop fibrotic tissue in such a manner that it will block the AqH outflow. This mechanism also could account for the fact that failure still occurs in cases long after GFS.

Variation between patients

Third, it is questionable whether the intraoperative MMC-treatment is equally effective in each individual, since MMC effectivity is p53- [32], pH- [18,24], and [O₂]- [26] dependent. Cheng *et al.* clearly showed that MMC deactivates Akt, which is known as a key player in cellular processes (such as glucose metabolism, apoptosis, cell proliferation, transcription, and cell migration), in a p53-dependent way, because Akt levels were only inhibited by MMC in MCF-7 (p53-proficient) cells and not in K562 (p53-deficient) cells [32]. Furthermore, Mudhol *et al.* [18] and Velpandian *et al.* [66] reported that MMC is inactivated in acidic solutions (pH of 6) and is mostly active at neutral pH (pH of 7). Most interestingly, in 1984, Coles and Jaros [67] reported that ocular surface pH shifted during the day and also differs between individuals. In addition, Verweij and Pinedo [26] reported that MMC is mainly active under anaerobic circumstances (low O₂ values, hypoxia). Most likely, this MMC activity under hypoxic conditions is an important reason why MMC is

effective in killing cancer cells, because it is generally known that cancer cells survive under hypoxic conditions [68,69]. In addition, as described in paragraph about 'MMC treatment is too short', the intraoperative MMC treatment and the washing techniques can differ between surgeries, which lead to different levels of MMC in the eye between individual surgeries [8]. Finally, patients differ also in fibrosis propensity due to for example differences in age, ethnicity, premedication, and genetic differences [16]. In conclusion, the inter-individual variability between patients regarding dose and activation of MMC as well as variations in the fibrosis process itself may result in variation in extent and timing of fibrosis and thus of failures between patients.

Toxic side effects

Fourthly, MMC treatment has several side effects. It is known that MMC is toxic to cells (e.g. hepatotoxicity) [36]. Systemic MMC treatment has several known side effects such as delayed myelosuppression, thrombocytopenia, leukocytopenia, and anemia [26,37]. Ocular use of MMC can lead to a variety of ocular complications mostly related to the typical thin-walled MMC filtering blebs, including blebitis, endophthalmitis, corneal epithelial defects, and hypotony with accompanying vision loss [10,18,33,49]. In conclusion, some failures may

result from toxic side effects of MMC. Furthermore, the mechanism of action of MMC is relatively harsh and unspecific, which means that it will also kill and inhibit cell growth of cells that would have a beneficial effect (such as the sub-conjunctival blood- and lymph-vessels that can transport/absorb the AqH of the filtering bleb).

Improvements of GFS medication regime

Clearly then, the current GFS medication regime is not fully effective in all patients. Roughly two different approaches have been taken to improve the success rate.

The first approach is to repeat or extend the duration of the administration of the currently used antifibrotic drugs. In fact, in case of failure or threat of failure, needle revisions (i.e. subconjunctival fibrotic strands are cut with a needle and an antifibrotic drug is applied again by subconjunctival injection) are often performed in the clinic. If necessary, this treatment can be repeated at various time points, sometimes long after surgery. For these needle revisions mostly 5-FU has been used, but also MMC needle revisions are performed, for example after a trabeculectomy and XEN gel stent surgeries [70–74,75[■],76–82]. Some examples of 5-FU and MMC needle revisions are shown in Table 1. It shows that needle revisions, with MMC or 5-FU of failing

Table 1. List of needle revisions with 5-fluorouracil (5-FU) or mitomycin C (MMC) (1–6; examples of needle revisions after trabeculectomy and 7–13; examples of needle revisions after XEN gel stent surgeries)

	Surgery procedure	Origin	Intraoperative treatment	Needle revision			Effect/success rates	Reference
				Drugs	Concentration	Volume		
1	Trabeculectomy	Human	MMC (0.4 mg/ml, 1–2 min)	5-FU	50 mg/ml	0.1 ml	34,3% (after 12 months)	[76]
				MMC	0.2 mg/ml	0.1 ml	57,5% (after 12 months)	
2	Trabeculectomy	Human	–	MMC	80 µg/ml	0.1 ml	76%	[77]
3	Trabeculectomy	Human	–	MMC	0.02%	0.15–0.2 ml	40,3% (after 3 years)	[75 [■]]
4	Trabeculectomy	Human	–	5-FU	50 mg/ml	0.1 ml	47%	[70]
				MMC	0.2 mg/ml	0.1 ml	80%	
5	Trabeculectomy	Human	–	MMC	0.3 mg/ml (60 µg)	0.2 ml	84.6% (after 2 years)	[78]
6	Trabeculectomy	Primates	MMC (0.27 mg/ml, 4 min)	–	–	–	–	[71]
				MMC	0.4 mg/ml	–	–	
7	XEN140 XEN63	Human	–	5-FU and MMC in 32% of cases			–	[82]
8	XEN140	Human	–	5-FU and MMC in 43% of cases			–	[81]
9	XEN45	Human	MMC	None			–	[79]
10	XEN45	Human	MMC	5-FU and MMC in 2.4% of cases			–	[72]
11	XEN45	Human	MMC	5-FU and MMC in 43.2% of cases			–	[80]
12	XEN45	Human	MMC	5-FU and MMC in 32.3% of cases			–	[74]
13	XEN45	Human	MMC	5-FU and MMC in 30.7% of cases			–	[73]

5-FU, 5-fluorouracil; MMC, mitomycin C.

Table 2. List of DDSs [1–9; examples of MMC loaded DDSs and 9–12; examples of DDSs with other drugs than MMC]

Name of device	Material	Type	Biodegradable?	Drugs	Concentration	Release period	Tested?	Reference
1 Nonbiodegradable poly (2-hydroxyethyl methacrylate) (P(HEMA))-mitomycin C	P(HEMA)	Disks/gel	No	MMC	0.17, 0.35, 0.8 mg MMC per gram gel	in vitro over 1 to 2 weeks (80% after 4 days)	In vitro and in vivo	[86]
2 Cyclodextrin polymers with MMC	Cyclodextrin polymers	Gel	Not tested (possibly long-term degradability)	MMC	6.5 µg	–	In vitro and in vivo	[87]
3 Biodegradable poly (lactic-co-glycolic acid) (PLGA) system with 5-fluorouracil (5-FU) with and without MMC	PLGA	Film	Yes	MMC	0.65 µg	–	In vitro and in vivo	[87]
Biodegradable poly (lactic-co-glycolic acid) (PLGA), burst of mitomycin C (MMC) release followed by a slow release of 5-fluorouracil (5-FU),					1 en 5 µg	20% after 8 days (1 µg) and 30% after 1 day (5 µg)	In vitro	[88]
4 Cross-linked hyaluronan (HA) hydrogel with MMC	HA	Hydrogel	Yes	MMC	0.1, 0.4, 2, mg	0.03 mg after 20 days (0.1 mg) and 0.24 mg after 20 days (0.4 mg) en 1.5 mg after 30 days (2.0 mg)	In vitro and in vivo	[31]
Cross-linked hyaluronan (HA) hydrogels that contained covalently-bound MMC		Gel			0.31%, 0.625%, and 1.25% of total film weight	70% after 4 days (thereafter no release anymore)	In vitro and in vivo	[89]
5 Biodegradable thermo-sensitive hydrogel from poly (trimethylene carbonate) 15-F127-poly (trimethylenecarbonate) 15 (PTMC15-F127-PTMC15) was designed and evaluated as an injectable implant. Loaded with MMC	PTMC15-F127-PTMC15	Hydrogel	Yes	MMC	0.1 mg/ml	80% after 15 days	In vitro and in vivo	[49]
6 A novel sustained release formulation of MMC based on RTGel, a proprietary thermosensitive hydrogel technology	MiroGel (thermosensitive hydrogel)	Hydrogel	?	MMC	2 mg/ml and 4 mg/ml	–	In vivo	[90]
			?		2 mg/ml (14 mg), 4 mg/ml (28 mg), and 8 mg/ml (56 mg)	–	In vivo	[91]

Table 2 (Continued)

	Name of device	Material	Type	Biodegradable?	Drugs	Concentration	Release period	Tested?	Reference
7	MMC encapsulated in LDLr targeting drug delivery system	LDL-MMC-chitosan nanoparticles (targeted)	Nanoparticles in HA film	Yes	MMC	–	60 h	–	[33]
8	Poly (3-hydroxybutyrate) (P(3HB)), containing the drugs mitomycin C (MMC) or paclitaxel (PTX)	P(3HB)	Film	Yes	MMC	69 µg of the 460 µg	3–4 days (80% released)	In vitro and in vivo	[92]
	Poly (4-hydroxybutyrate) (P(4HB)), containing the drugs mitomycin C (MMC) or paclitaxel (PTX)	P (4HB)		Yes	PTX	213 µg of the 1418 µg	25 days (1% released)		
					MMC	63 µg of the 420 µg	3–4 days (80% released)		
					PTX	112 µg of the 747 µg	25 days (14% released)		
9	Lipidated prodrug of mitomycin C (MLP) on the membrane of a pegylated liposome formulation (PL-MLP), also known as Promitil	Membrane of a pegylated liposome formulation (PL-MLP), also known as Promitil	Liposome	Not tested (likely Yes)	MLP (MMC)	5 mg/ml	–	In vitro	[93]
10	Polycaprolactone (PCL) implants with a proprietary ocular hypotensive agent (DE-117)	PCL	Film	Yes	DE-117	–	180 days a constant release of 0.5 µg/day	In vitro and in vivo	[94]
11	Poly (lactic-co-glycolic acid) (PLGA) comprising cyclosporine A (CsA)	PLGA	Coating/film	Yes	CsA	6 mg	80% after 100 days, the best and highest release are found after the first 10 days	In vitro and in vivo	[95]
12	Electrospun fibers were prepared with bevacizumab encapsulated in the core, surrounded by a poly-e-caprolactone sheath.	Electrospun fibers surrounded by a poly-e-caprolactone sheath	Fibers reservoir	Not tested (unlikely that polymer would undergo complete degradation)	Bevacizumab	24 µg bevacizumab in 1 mg of fibers	60% after 20 days	In vitro	[96]

5-FU, 5-fluorouracil; DDS, drug-delivery system; LDLr, low density lipoprotein receptor; MMC, mitomycin C.

filter blebs after trabeculectomy, are successful [70,71,75^a,76–78]. Furthermore, it shows that a needle revision with MMC is more effective than a needle revision with 5-FU [70,76]. Needle revisions, with 5-FU or MMC, are needed in 30% to 50% of all the XEN gel stent surgery cases [72–74,79–82].

The positive results of repeated administration of MMC suggest that the onetime intraoperative application of MMC is too short to curtail fibrosis in the long term. It may be better to present antifibrotic drugs by a drug delivery system (DDS) for sustained drug delivery. This sustained delivery may result in a longer and better (better controlled in dose and duration) MMC treatment than the current onetime intraoperative MMC treatment [13,83,84]. In this manner, the antifibrotic drugs are present throughout the whole wound healing process. Furthermore, a DDS with continuous delivery of MMC over a long time, may be more efficacious since fibroblasts and/or tissue are continuously exposed to AqH that contains inflammatory cytokines and an important growth factor for the inducement of the wound healing processes. Due to the long-term postoperative scar formation time, which lasts at least for over 2 weeks [85], a DDS that can maintain an effective drug concentration for an appropriate period of time to regulate postoperative proliferation and maintain bleb filtering with fewer complications is desirable [49]. Multiple sustained drug delivery systems (DDSs) have been developed in the past years. Many of these studies with the DDSs were performed within the eye of humans and/or animals. Examples of interesting DDSs are listed in Table 2: (1) a nonbiodegradable poly (2-hydroxyethyl methacrylate) (P (HEMA))-MMC device [86,87], (2) cyclodextrin polymers loaded with MMC [83], (3) a biodegradable poly (lactic-co-glycolic acid) (PLGA) system with 5-FU with and without MMC [87,88], (4) a cross-linked hyaluronan (HA) hydrogel that contained MMC [31,89], (5) a thermo-sensitive hydrogel from poly (trimethylene carbonate)15-F127-poly (trimethylene carbonate)15 (PTMC15-F127-PTMC15) loaded with MMC [49], (6) a thermosensitive hydrogel from MitoGel loaded with MMC [90,91], (7) MMC encapsulated in low density lipoprotein receptor targeting DDS (LDL-MMC-chitosan nanoparticles) [33], (8) thin films of two different polymers, Poly (3-hydroxybutyrate) (P (3HB)) and Poly (4-hydroxybutyrate) (P(4HB)), containing MMC or paclitaxel (PTX) were attached to silicone-tubes [92], (9) lipidated prodrug of MMC (MLP) on the membrane of a pegylated liposome formulation (PL-MLP), also known as Promitil [93], (10) polycaprolactone implants that achieve zero-order release of a proprietary ocular hypotensive agent (DE-117) [94], (11) a PLGA

system loaded with cyclosporine A [95], and (12) encapsulation of bevacizumab in the core of electrospun fibers [96]. No DDS for sustained delivery of antifibrotic drugs is currently FDA approved. One DDS of MMC, namely PL-MLP (see also in Table 2), is now being tested in clinical trials.

The second approach to improve GFS outcome in the long term is to develop other (classes of) GFS medication [4,15,16]. This option will not be discussed in detail in this review. Multiple drugs have been tested in the past years, often aiming to influence the wound healing after GFS in a more specific and physiological manner (e.g. by targeting TGF- β) rather than the unspecific treatment with MMC. This research has extensively been reviewed by Li *et al.* [14], Schlunck *et al.* [9], Yu-Wai-Man and Khaw [4], and Friedlander [97]. These new GFS drugs may avoid several of the side effects of MMC and maybe also the individual variability between patients. In the future, medication may be personalized taking account of individual differences in fibrosis [98].

CONCLUSION

GFS is currently the last resort treatment to stop or limit vision loss in patients that suffer from glaucoma. A major complication, which frequently causes failure of GFS, is the formation of fibrosis in the filtering bleb. The antifibrotic drug MMC, given as an adjuvant during GFS, has significantly increased GFS success rates. MMC is also used in GFS surgery with the novel minimally invasive glaucoma surgical (MIGS) devices.

Although MMC certainly improved GFS success rates, still roughly 10% of bleb-based MIGS surgeries with a onetime MMC application fail within the first year, mostly because of fibrosis in the filtering bleb. To further reduce GFS failure rate, we roughly have two options: either we improve the MMC application or we switch to new drugs. Treatment of GFS failures with repeated injections of MMC shows positive results. This suggests that prolonged presence of MMC may control fibrosis over a longer period of time. DDSs for sustained release of MMC are currently being developed and these will show if this hypothesis is true. If not, we need to implement new approaches and drugs to create a suitable GFS wound healing, which will further increase GFS success rate.

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Conflicts of interest

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- of special interest
- of outstanding interest

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