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Review Article

Engineered microorganisms: A new direction in kidney stone prevention and treatment

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ABSTRACT

Numerous studies have shown that intestinal and urinary tract flora are closely related to the formation of kidney stones. The removal of probiotics represented by lactic acid bacteria and the colonization of pathogenic bacteria can directly or indirectly promote the occurrence of kidney stones. However, currently existing natural probiotics have limitations. Synthetic biology is an emerging discipline in which cells or living organisms are genetically designed and modified to have biological functions that meet human needs, or even create new biological systems, and has now become a research hotspot in various fields. Using synthetic biology approaches of microbial engineering and biological redesign to enable probiotic bacteria to acquire new phenotypes or heterologous protein expression capabilities is an important part of synthetic biology research. Synthetic biology modification of microorganisms in the gut and urinary tract can effectively inhibit the development of kidney stones by a range of means, including direct degradation of metabolites that promote stone production or indirect regulation of flora homeostasis. This article reviews the research status of engineered microorganisms in the prevention and treatment of kidney stones, to provide a new and effective idea for the prevention and treatment of kidney stones.

1. Introduction

Kidney stones are one of the most common diseases of the urinary system, among which calcium oxalate stones are the most common, accounting for about 75%–85% of all urinary stones [1,2]. In recent years, with the development of lithotripsy technology, the stone free of patients with kidney stones has improved significantly after surgery, but unfortunately there is no effective way to prevent stone recurrence. Epidemiological studies have shown that the incidence of nephrolithiasis has increased in recent decades, with a 5-year recurrence rate as high as 50%. Despite decades of research, treatment options to prevent relapse remain limited.

Previous studies have primarily examined the impact of metabolic factors, such as hypercalciuria and hyperoxaluria, on the formation of stones. However, there has been a growing interest in the role of microorganisms, including those found in the gut and urinary tract, in stone formation in recent years. Dysbiosis, is a crucial factor in the formation and advancement of kidney stones. Several studies have demonstrated the potential of adjusting the human microbiota to prevent and treat kidney stones [3,4]. As a result, microbial-based therapies have the potential to revolutionize stone prevention.

In healthy individuals, specific probiotics play a vital role in maintaining good health by aiding in the digestion of food, producing beneficial metabolites like short-chain fatty acids (SCFAs), degradation of harmful metabolites such as oxalate, and treating inflammatory diseases such as colitis [5–7]. Probiotic supplementation is one of the common strategies to restore microbial ecological dysbiosis and maintain microbial homeostasis. Despite their potential benefits, conventional probiotic treatments have demonstrated certain drawbacks. These include the transfer of antibiotic resistance genes, the production of harmful metabolites, and a lack of significant action in the intestine. Additionally, conventional probiotics may fail to produce specific substances necessary for the treatment of certain diseases [8,9].

In recent years, there has been a growing interest in the design of

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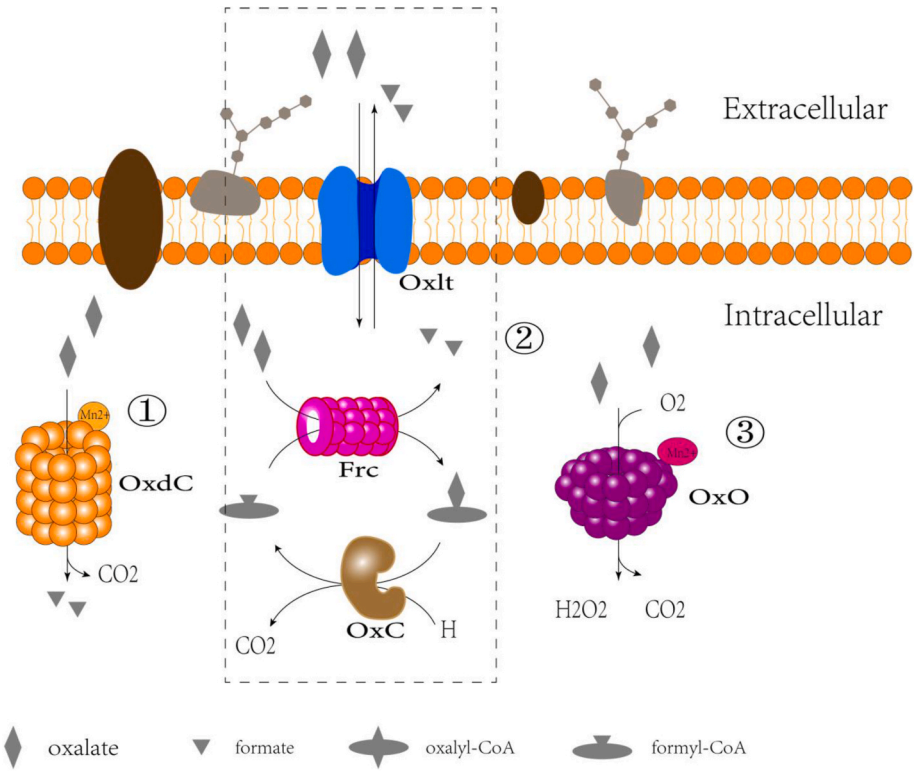

Fig. 1. The most common oxalate degradation pathways in current research.

probiotics with specific characteristics using a strategy of engineered microorganisms. This approach utilizes genetic tools to cut, splice, and integrate target genes, which are then introduced into the host bacterium to create a new phenotype or enhance its ability to express heterologous proteins. Recent studies have shown that genetically engineered bacteria and their products have the potential to treat various diseases through oral or intravenous administration [10,11]. These bacteria can provide therapeutic benefits by producing active molecules, interfering with immune cells, inhibiting pathogenic bacteria, or expressing exogenous genes in a variety of ways [12,13]. This review aims to provide new ideas for the prevention and treatment of kidney stones by summarizing and discussing published articles on modifying intestinal and urinary flora. Specifically, the focus is on degrading metabolites and regulating dysbiosis as a means of preventing kidney stone occurrence.

2. Intestinal engineered microorganisms

1. Oxalate-degrading bacteria in the intestine.

Oxalate is produced as a result of human metabolism. The urine contains oxalate from two main sources: endogenous oxalate produced during liver metabolism and dietary oxalate absorbed in the intestine. Enteric hyperoxaluria, which is caused by excessive absorption of oxalate in the intestine, is a significant risk factor for the formation of calcium oxalate stones. Higher intake and absorption of oxalate can lead to a significant increase in urinary oxalate excretion [14–16]. In healthy populations, the synthesis of oxalate in the liver and the absorption of oxalate in the gastrointestinal tract from the diet contribute equally to the total urinary oxalate excretion. However, pathologically elevated urinary oxalate concentrations may be more associated with increased gastrointestinal oxalate absorption [17–20]. Hyperoxaluria is a significant risk factor in the formation of calcium oxalate kidney stones as urinary calcium concentrations are typically 10 times higher than oxalate. Even minor changes in urinary oxalate concentrations can significantly impact the saturation of calcium oxalate crystals [18,21]. Thus, reducing urinary oxalate concentration is a crucial preventive and treatment strategy for oxalate-related stones.

Oxalate is absorbed throughout the entire intestinal tract, but a significant portion of dietary oxalate is absorbed in the upper part of the intestine [22,23]. The colonic mucosa has a high permeability, making it a primary location for increased oxalate absorption. Additionally, oxalate-degrading bacteria, such as Oxalobacter formigenes, which require a strictly anaerobic environment to survive, tend to colonise in the colon [24–26].

To prevent kidney stones, one approach is to decrease the consumption of foods that are high in oxalate [27–29]. However, limiting the intake of oxalate-rich foods like spinach to reduce the recurrence of stones may result in deficiencies in essential nutrients, and some patients may struggle to comply with this recommendation [16]. Therefore, attention has gradually shifted to oxalate-degrading microorganisms, which can degrade oxalate and possibly regulate intestinal oxalate secretion [30,31]. The colonization of oxalate-degrading bacteria, particularly Oxalobacter formigenes in the gut, contributes to the degradation of oxalate in food and reduces intestinal absorption and urinary oxalate excretion, subsequently reducing the risk of calcium oxalate stones [32–36]. However, oxalate-degrading bacteria, represented by Oxalobacter formigenes, are strictly anaerobic bacteria that are highly sensitive to antibiotics and require a high oxalate diet for colonization, which increases their difficulty in clinical applications [37,38]. Some other natural intestinal oxalate-degrading bacteria, such as Lactobacillus and Bifidobacterium, have shown inconclusive oxalate-degrading effects in vitro and mouse experiments [39]. Meanwhile, several subsequent studies in humans have shown that the efficacy of the use of natural probiotics to reduce urinary oxalate concentrations remains highly controversial [40–42]. Therefore, considering the structure of the human gut and the complexity of gut microorganisms, the use of engineered bacteria that are more likely to survive and colonise the gut carrying an oxalate-degrading system (Fig. 1) so that they can more efficiently express oxalate-degrading enzymes and thus exert their oxalate-degrading capacity may be a good

strategy to address this issue.

①Bacillus subtilis degrades oxalate to form formic acid and carbon dioxide through OxdC ②Oxalobacter formigenes represents oxalate degradation to formic acid and carbon dioxide through OxlT, OxC, and Frc ③Oxalate is oxidised to carbon dioxide by OxO in barley.

(1) Engineered of the oxalate degrading genetic flora of Oxalobacter formigenes

Some members of the human intestinal microbiota have the ability to break down oxalate in the gut, such as Oxalobacter formigenes, which possesses an oxalate degradation pathway that could provide a new direction for enterogenic hyperoxaluria: consisting of a protein-encoding oxalate-formate reverse transporter (OxlT), oxalyl-coenzyme A decarboxylase (OxC) and formyl-coenzyme A transferase (Frc). 1. OxlT transports oxalate into the cytoplasm in exchange for out formate in the cell; 2. Frc facilitates the transfer of coenzyme A (CoA) from formyl-CoA to oxalate to produce oxalyl-CoA and formate; 3. OxC promotes the production of formyl-CoA and carbon dioxide from oxalyl-CoA, regenerating the substrate required for this cyclic reaction.

Oxalobacter formigenes has been a hot topic in the treatment of calcium oxalate kidney stones caused by hyperoxaluria since its discovery in the 1980s. The lack of oxalate-degrading bacteria in the digestive tract is one of the risk factors for hyperoxaluria and kidney stones, and a case-control study of 274 patients with recurrent calcium oxalate stones and 259 age- and sex-matched normal subjects also demonstrated significantly lower colonization rates of Oxalobacter formigenes in patients with stones [43–45]. Interestingly, in subsequent studies, it was found that Oxalobacter formigenes may not only degrade oxalate in the gut and thus reduce absorption, but may also secrete a bioactive substance that can be inactivated by heat or pepsin and interact with the intestinal epithelium to promote intestinal oxalate secretion, particularly in patients with chronic renal failure [24,46,47]. Some past animal studies have claimed that Oxalobacter formigenes can reduce urinary oxalate concentrations in hyperoxaluria mice, and subsequent clinical trials with small samples have confirmed its usefulness in patients with hyperoxaluria [42,48]. However, in a recent series of randomised controlled trials of oral lyophilised capsules of Oxalobacter formigenes in patients with primary hyperoxaluria, Oxalobacter formigenes intake did not reduce urinary oxalate concentrations and only a small number of patients were able to achieve colonization with Oxalobacter formigenes [35,49,50]. A meta-analysis of all studies on Oxalobacter formigenes revealed that the bacterium's ability to degrade urinary oxalate was not essential nor adequate [51]. Nonetheless, current research confirms that Oxalobacter formigenes is capable of breaking down oxalate as a probiotic that specializes in oxalate as a metabolic substrate. However, the primary challenge is colonizing the gastrointestinal tract to act as an oxalate degrader and secretion promoter. The fragility of Oxalobacter formigenes poses a number of problems in the production and packaging processes, and the poor environment in the gastrointestinal tract means that only a small proportion of active Oxalobacter formigenes reaches the gastrointestinal tract, making colonization and oxalate degradation difficult to achieve [52]. At the same time, the use of antibiotics can also contribute to the loss of oxalate bacilli colonization in the body, which in turn is not effective in reducing oxalate in the long term for the prevention and treatment of calcium oxalate kidney stones [53,54]. Therefore, the use of less vulnerable probiotic bacteria carrying oxalate degrading systems into the gut to exert oxalate degrading capacity may be a good solution strategy.

OxC and Frc genes are thought to play a central role in oxalate degradation, however,Lactobacillus, which can express OxC and Frc genes, can not effectively degrade oxalate, which may be related to the lack of oxalate transporters [55]. In a subsequent study, Nissle 1917, which expresses OxlT, OxC and Frc, also failed to detect oxalate degradation, and the addition of an oxalyl coenzyme A synthase gene from *Saccharomyces cerevisiae* (scaaE3) showed strong oxalate degradation, which was demonstrated in subsequent in vitro gastrointestinal simulation (IVS) systems and in experiments with mice and non-human primates [3]. Further Phase I clinical trial in human will also demonstrate the reliability and safety of this recombinant Probiotic. Notably, it has been claimed that succinate in bacteria is a better coenzyme A receptor than oxalate and that succinyl coenzyme A synthesized by Frc may disrupt the oxalate degradation cycle by depleting formyl CoA in bacteria, while the use of formyl CoA transferase, which is more specific for oxalate, might be a good solution strategy [56].

(2) Engineered of oxalate decarboxylase-associated bacterial flora

Oxalate decarboxylase is an effective enzyme found in Bacillus subtilis that is capable of degrading oxalate even in low pH environments. Probiotic strains expressing OxdC have been developed in various studies and have demonstrated successful oxalate degradation in both in vitro and in vivo experiments conducted in the intestine [57–60].

Professor Govindan Sadasivam Selvam's group has been working on the modification of oxalate decarboxylase-related engineered bacteria. First, they developed a lactic acid bacterium heterologously expressing the Bacillus subtilis OxdC gene to consume oxalate in the intestine using Lactobacillus plantarum NC8 and the pSIP expression system [58]. They then used the lactate dehydrogenase promoter (PldhL) of Lactobacillus plantarum NCIMB8826 to enhance OxdC expression in recombinant Lactobacillus plantarum NC8, and after incubation in 50 mmol/L sodium oxalate MRS broth for a period of time detected up to 90% oxalate degradation in the recombinant bacteria, compared to 15% in the corresponding plasmid-containing and wild-type Lactobacillus plantarum [60]. To promote OxdC secretion into the intestinal lumen, they used promoter elements Lp_0373 and Lp_3050, which carry the secretion signal, to drive OxdC protein expression and secretion in recombinant lactic acid bacteria WCFS1 [61]. As a result of these experiments, non-secretory recombinant lactic acid bacteria (NC8OxdC) and secretory recombinant lactic acid bacteria (WCFS1 OxdC) were obtained and tested in vivo in rats, showing that they both increased the degradation of oxalate in the intestine and thus reduced the excretion of oxalate in the urine and the deposition of calcium oxalate crystals in rats [59]. Recently, they integrated the OxdC gene into the chromosomal thymidylate synthase (thyA) gene of Lactobacillus plantarum WCFS1 via the mobile group II intron, thus overcoming the loss of the recombinant bacterial plasmid and the presence of antibiotic-resistance genes [62]. At the same time, this modified strain will die in the absence of thymidine in the environment due to the disruption of the chromosomal thymidylate synthase (thyA) gene, thus acting as a biological containment. Meanwhile, Professor Chen also conducted some exploration of the OxdC gene [63], using the acid-inducible promoter p170 to enhance the expression of the OxdC gene in Lactobacillus MG1363, and recombinant Lactobacillus was also effective in degrading oxalate, reducing hyperoxaluria and inhibiting calcium oxalate stone formation in both in vitro and in vivo experiments.

The heterologous expression of the OxdC gene in Lactobacillus for oxalate degradation appears to be feasible, but again, the possible lack of oxalate transporters on the surface of the bacteria needs to be overcome, and few previous studies have shown that Lactobacillus can achieve the high throughput of oxalate transport. If oxalate is not replenished and the degradation product formic acid is not transported out of the cell in time, then the oxalate degradation capacity and clinical utility of this bacterium will be greatly reduced. Would the use of oxalate transporter proteins to degrade oxalate in a more favourable intracellular environment be more effective than the use of promoter elements carrying secretory signals to enable OxdC to work in the gut? But unfortunately, no one has ever considered the combination of OxdC and OxlT for oxalate degradation and whether their ability to degrade oxalate could be contested and these need to be confirmed by researchers.

In addition, numerous studies have shown that oral administration of

OxdC rather than recombinant bacteria to degrade oxalate in the gut appears to be a good option. In a group of experiments designed on AGT1 knockout mice (AGT1KO, which causes hyperoxaluria in mice), AGT1KO mice were given four different doses of OxdC preparations or a placebo mixed with food [64]. The results showed a 44% and 72% reduction in oxalate in the urine and faeces of AGT1KO mice treated orally with the 200 mg OxdC preparation compared to the placebo. At the same time, each of the other three doses of OxdC reduced urinary oxalate excretion by more or less 30–50% in the AGT1KO mice, while in the 80 mg group, urinary oxalate excretion was consistently reduced by more than 40%, with a 100% survival rate and effective prevention of renal calcium deposits and kidney stones. It is worth noting that this OxdC preparation was active between pH 3.0 and 7.5, which allowed it to exert oxalate degradation at various locations in the digestive tract. In another a double-blind, placebo controlled, randomized Phase 1 cross-over study with ALLN-177, a Bacillus subtilis oxalate decarboxylase expressed orally in recombinant *Escherichia coli*, urinary oxalate excretion was significantly reduced in subjects given oral ALLN-177 compared to placebo, resulting in a 17% reduction in urinary oxalate excretion in subjects [65]. Meanwhile, Professor Craig [66] conducted a clinical study using ALLN-17 in subjects including 5 subjects with enterogenic hyperoxaluria and 11 subjects with idiopathic hyperoxaluria and showed that after treatment with ALLN-177, the overall mean (SD) urinary oxalate excretion decreased from 77.7 mg/24h before treatment to 63.7 mg/24h, a mean reduction of 14 mg/24h. Another prospective, double-blind, randomised, placebo-controlled, crossover study of oral administration of oxalate decarboxylase (OxdC) expressed in recombinant *Escherichia coli* also showed a significant reduction in urinary oxalate following OxdC treatment, with a reduction in urinary oxalate excretion of 12.5 mg or 29% ($p < 0.001$) at 24 h after 4 days of treatment compared to pre-treatment [67].

The ability of OxdC to degrade oxalate in the gut and thereby reduce urinary oxalate excretion in the kidney and prevent the development of renal calcium oxalate stones has been demonstrated in a series of in vivo and in vitro experiments. However, OxdC is a protein that cannot be synthesized in humans and its safety in the intestine has been a concern for researchers and physicians. In a set of fourteen-day animal studies on the safety of mutant OxdC [68], rats and dogs were given doses of mutant oxalate decarboxylase greater than 50 times the clinically expected dose after assessing daily food intake, body weight, clinical chemistry, urinalysis, haematology and some histopathological (kidney, large and small intestine, stomach, liver, heart, testis, lung, spleen and thyroid) specimens and found that both No serious adverse reactions or deaths associated with the test specimens occurred in either animal. This indirectly demonstrates the safety of the heterologously expressed OxdC enzyme in the intestine [57,65], but the safety and long-term efficacy of OxdC in the intestine remains questionable and needs to be confirmed in more animal studies and clinical trials.

The ileum and colon are the main areas of oxalate absorption and secretion, with a pH = 7 [57,69]. Natural oxalate-degrading bacteria have a strong oxalate degradation capacity when the ph is between 5 and 6, whereas the efficiency of oxalate degradation decreases sharply at ph > 6 [70–72]. Meanwhile, in another report, Bacillus subtilis oxalate decarboxylase obtained from recombinant E. coli was reported to have the highest oxalate degrading activity at pH 5, and the enzyme activity was already reduced at pH 7 or at 37 °C. These factors may limit the use of these probiotics, but it has also been claimed that OxdC activity under neutral conditions in the gut, even though it has declined, is still able to achieve the desired degradation of urinary oxalate [57]. Meanwile, if these bacteria can be treated with genetically modified elements or synthetic biology proteins to show good oxalate degradation efficiency even under neutral conditions, it is expected that these problems can be solved to effectively degrade oxalate in the gut to reduce urinary oxalate excretion and ultimately inhibit stone formation.

(3) Engineered of oxalate oxidase-related bacterial flora

Oxalate oxidase catalyzes the oxidation of oxalate to carbon dioxide and hydrogen peroxide. In the past, Chen et al. [63] heterologously expressed oxalate oxidase from barley in the lactic acid bacterium MG1363 and used the acid-inducible promoter p170 to enhance the expression of the oxalate oxidase gene. Yet a series of in vitro and in vivo experiments showed that its oxalate degradation capacity was almost negligible. It is no coincidence, however, that some previous experiments have attempted to heterologously express barley oxalate oxidase in E. coli, but unfortunately, none of these studies detected the expression of active oxalate oxidase [63,73].

It is generally believed that the reducing environment in the cytoplasm of E. coli is not conducive to the formation of disulfide bonds in proteins, making it an unsuitable host for the expression of oxalate oxidase stabilized by disulfide bonds [74]. Heterologous proteins containing disulfide bonds produced by E. coli are often degraded or misfolded to form insoluble aggregates called inclusion bodies [75]. Oxalate oxidase expression and functional expression may be achieved to some extent by lowering the culture temperature, co-expression using chaperones or folding enzymes and fusion with highly soluble proteins, while the use of the recently developed trxB gor double mutant lacking thioredoxin reductase and glutathione reductase as an expression host is also a good coping strategy, with its relatively oxidative state The intracellular environment allows for disulfide bond formation by oxalate oxidase in the cytoplasm [76].

Dahiya et al. [77] used liposome-encapsulated oxalate oxidase in a rat model to reduce urinary oxalate excretion with success, suggesting that oxalate oxidase is capable of oxalate degradation in the intestine, but requires a suitable internal environment to facilitate disulfide bond formation in order to exert its oxalate degradation This requires reprocessing of its expressed proteins based on a range of synthetic biology knowledge, which may be fruitful. At the same time, the heterologous nature of these proteins, which may be digested by pepsin and cause some immune reactions, makes oxalate oxidase difficult to use in clinical work. Nevertheless, Nevertheless, oxalate oxidase, an oxalate-degrading enzyme that uses oxygen to degrade oxalate to carbon dioxide and hydrogen peroxide, has shown promise in the prevention and treatment of enteric hyperoxaluria and calcium oxalate stones if the latest synthetic biology knowledge can be used to overcome these problems.

2. Regulation of metabolism to achieve flora homeostasis

To date, the microbiota has been associated with a range of diseases such as neurological disorders, inflammatory diseases and cancer, while certain gut microbiota produce metabolites that are important for maintaining a healthy host gut homeostasis [78–82]. The gut microbiota plays an important role in entero-renal pathophysiology and can be used to maintain oxalate homeostasis in healthy populations through a multi-species bacterial network to inhibit kidney stones [83]. Recent studies have shown that oxalate metabolism is driven by a diverse network of oxalate-degrading microorganisms that can be transferred between mammalian species and can significantly and consistently reduce urinary oxalate excretion more effectively than the use of oxalate-degrading bacteria alone [84].

Although the human gut microbiota is a very complex community, there are a number of strategies that can be used to try to influence its composition and function [85,86]. One common approach is to use naturally occurring probiotics in the gut that are thought to be beneficial to the host, but their effects on the host and other microbiota in the gut are not fully understood and evidence of their ability to effectively colonise the gut remains scarce [87,88]. Based on the current dilemma facing the natural intestinal flora, supplementation with probiotics that can express beneficial effects on intestinal flora homeostasis is slowly becoming a hot topic of research today. For example, SCFAs (short-chain fatty acids), have been shown in several studies to be associated with the dysregulation of SCFA metabolism and various diseases in humans

[89–91], and SCFAs produced by microorganisms in the gut can inhibit the development of kidney stones through various modulatory mechanisms [92–95]. Heterologous expression of SCFAs in Bacillus subtilis has a role in regulating metabolic disorders and maintaining intestinal flora homeostasis in mice [96].

Colitis is a common disease of the colon that can lead to enterogenic hyperoxaluria and kidney stones and is often difficult to treat clinically [97]. Chen et al. [98] used E. coli Nissle 1917 (EcN) as a vector to design a probiotic that heterologously and continuously expressed SCFAs in the intestinal lumen of mice with colitis, which was effective in improving the colonic microenvironment. The probiotic was effective in improving the colonic microenvironment and producing a therapeutic effect on colitis. Similarly, a growing number of different engineered intestinal bacteria for the treatment of colitis have been used successfully, with recombinant probiotics being able to improve the intestinal microenvironment, regulate intestinal homeostasis and treat colitis in a variety of ways [11,99–101]. These modifications may also be important breakthroughs in the prevention or treatment of kidney stones caused by colitis-mediated hyperoxaluria.

3. Engineered of flora associated with other types of stones than calcium oxalate stones

Uric acid stones account for about 9% of all types of stones [102]. The production and excretion of uric acid are normally balanced in humans, but inadequate excretion or overproduction of urate for a number of reasons can lead to increased urate concentrations in the extracellular fluid. When urate concentrations reach their limit, deposits of urate crystals may occur, causing gout and uric acid stones. While mammals in nature are widely endowed with uric acid degrading enzymes, the gene encoding uric acid degrading enzymes in humans has been inactivated during human evolution, making uric acid the end product of purine metabolism in humans.

Pegloticase, a recombinant mammalian uricase expressed in *Escherichia coli*, is now commonly used to catalyse the oxidation of uric acid to a more soluble form of allantoin [103,104]. Intravenous Pegloticase has a potent and long-lasting uric acid-lowering capacity, with rapid efficacy in the regression of gout stones [104–106]. However, despite prophylactic treatment with high-dose steroids prior to injection, the range of infusion-related reactions that occur with Pegloticase treatment and the production of associated antibodies severely limit its use in clinical practice [107–109].

Pegloticase is an exogenous protein relative to the human body and systemic injections are often accompanied by a range of systemic symptoms. This severely limits the clinical use of this enzyme. Studies have shown that in a healthy population, approximately two-thirds of uric acid is excreted by the kidneys and one-third is secreted into the gut for bacterial degradation or elimination by faeces [110–112]. This gives us an insight into whether reducing urinary uric acid load by increasing uric acid degradation in the gut can prevent and treat the development of urinary stones when the total amount of uric acid produced systemically remains unchanged, and expressing uricase in the gut may be a good coping strategy.

Kateryna et al. [113] cloned a DNA fragment of a Candida uricase mutant into an expression vector and then expressed it in *Escherichia coli*. A series of screens yielded an optimal trypsin-resistant mutant and modified it named ALLN-346, and subsequent animal experiments also confirmed that oral administration of ALLN-346 resulted in normal uric acid excretion in mice. Unlike existing therapies containing uricase injections (which can cause infusion reactions and allergic reactions), ALLN-346 reduces the systemic urate load by degrading uric acid in the gut in order to reduce renal uric acid excretion and the potential for uric acid stones, and is therefore less likely to cause serious systemic reactions. The use of genetically engineered probiotics to express uricase in the gut may be a good alternative to the direct degradation of uricase in the gut. On the one hand, it ensures that the enzyme degrades uric acid in a relatively stable intracellular environment, and on the other hand, avoiding direct contact between the intestine and uricase can make the treatment safer.

3. Urethral engineered microorganisms

Several studies have shown that the urinary tract is not sterile in the absence of any symptoms of urinary tract infection [114]. The naturally occurring lactic acid bacteria in the urinary tract provide an excellent platform for the treatment of kidney stones, opening up a new avenue for the treatment of kidney stones by the human microbiota. The urinary microbiota is associated with kidney stones and may play a more important and direct role in the pathogenesis of kidney stones than the intestinal microbiota [115,116]. Unfortunately, research on urinary microbes is in its infancy and relatively little has been done on them, but because they are often in direct contact with various components of the urine, they have an irreplaceable potential in the prevention and treatment of kidney stone development.

1. Controlling urinary tract infections

Infection stones account for approximately 15% of all stone types in the urinary tract [117]. However, bacterial resistance to existing antibiotics is developing at an alarming rate, and the formation and prevalence of multidrug-resistant pathogenic bacteria presents new challenges in the treatment of urinary tract infections. In the past, attempts have been made to competitively inhibit multidrug-resistant pathogenic bacteria by colonizing the urinary tract of patients with recurrent urinary tract infections with an E. coli 83972 isolated from asymptomatic infected patients with success, while in another group of studies with E. coli HU2117 (a derivative of E. coli 83972) showed that colonization with HU2117 did not prevent the colonization and infection of pathogenic bacteria, but that urinary tract microbial diversity may have a protective role in invasive infections [118–120].

Due to the limitations faced by conventional urinary tract probiotics, several molecular biology techniques are being applied to understand the mechanisms of bacterial resistance and to develop new drugs against drug-resistant bacteria [121–123]. The use of genome editing tools to engineer new biosynthetic pathways in the microbial host has proven to be an ideal strategy for the production of novel antibiotics. In a recent study, a non-pathogenic strain of Mycoplasma pneumoniae that effectively degrades *Staphylococcus aureus* biofilms in human catheters was engineered to secrete antimicrobial enzymes that target bacterial biofilms for bactericidal purposes [124]. In other similar experiments, using Lactobacillus as an expression vector, researchers induced the expression of three antimicrobial substances: enterocin A, hiracin JM79 and enterocin P by recognizing the pheromone cCF10 produced by enterococci, and further studies showed that this recombinant bacterium could effectively inhibit the growth of multi-drug resistant enterococcal strains [125]. Sriram Seshadri et al. [126,127] also used Lactobacillus DT24 isolated from the vagina as an expression vector to express heterologous E. coli to inhibit uropathogenic E. coli.

The use of recombinant probiotics to inhibit the causative organisms of recurrent urinary tract infections is considered to be one of the strategies for the prevention and treatment of infectious stones. A recombinant *Escherichia coli* capable of recognizing *Pseudomonas aeruginosa* in the urinary tract was designed to recognize and secrete an antimicrobial agent to inhibit the growth of pathogenic bacteria for the purpose of controlling urinary tract infections [128]。

A recombinant E. coli expressing P fimbriae oligosaccharide receptor mimic (a strain isolated from the urinary tract of asymptomatic infected patients) 83972 was designed to effectively inhibit the adhesion and colonization of pathogenic bacteria, never forming a protective effect on the urinary tract [129]. Similarly, two recombinant Lactococcus lactis have been designed to antagonize the adhesion and biofilm formation of pathogenic bacteria, thereby inhibiting the occurrence of urinary tract

infections to some extent [130,131]. Although the above experiments were successful in vitro, further experiments are needed to determine whether these exogenous substances, in addition to pathogenic bacteria, cause damage to the intrinsic flora of the urinary tract and whether they are safe.

In contrast to the use of exogenous substances to inhibit the adhesion and colonization of pathogenic bacteria, Paola Scavone et al. [132,133] used recombinant Lactococcus lactis to colonise both the nasal and urinary tract mucosa and to secrete the MrpA bacteriophage protein of *Proteus mirabilis*, conferring a specific immune response to *Proteus mirabilis* in the host. Studies have shown that colonization of recombinant Lactococcus can effectively reduce the colonization of *Proteus mirabilis* in the urinary tract of mice, and can produce a series of antibodies against the MrpA pili protein of *Proteus mirabilis*.

Unfortunately, research into targeting infection-causing stones through engineered microorganisms is still at an exploratory stage, with a variety of approaches emerging, but further research is needed to determine whether these recombinant probiotics in vivo compromise the inherent flora of the urinary tract and whether the diversity of the urinary tract flora will be disrupted. However, the use of probiotics rather than increased doses of antibiotics or the use of other types of antibiotics to intervene in complicated urinary tract infections may be an important solution in the face of future epidemics of multidrug-resistant bacteria, and all of these success stories offer fresh ideas for the future management of infectious stones.

2. Homeostatic regulation of urinary flora

In healthy individuals, the urinary tract is not sterile and the various bacteria in the genitourinary tract are known as the urobiota. Studies have shown that ecological dysbiosis of the urinary microbiota is associated with various urological disorders [134,135]. Urinary microbiota is closely related to the development of stones, that the diversity of the urinary microbiota is significantly lower in patients with kidney stones than in healthy controls, and that an imbalance in the balance of the urinary flora may contribute to the development of stones [136]. A study showed that dysbiosis of the urinary flora preceded the development of urinary tract infection, suggesting that dysbiosis of urinary flora homeostasis may play an important role in the development of infectious stones [137]. Notably, antibiotics commonly used in the past for the prevention and treatment of kidney stones may have led to the loss of colonization of some health-protective lactic acid bacteria and their subsequent occupation by enterobacteria, thus promoting the development of kidney stones [115,138,139].

In the intestinal flora we already know that oxalate metabolism is constituted by a diverse network of oxalate-degrading microorganisms, and whether the urinary microflora has a similar oxalate degrading effect thereby reducing the incidence of urinary calcium oxalate stones has not yet been studied to confirm them, but it has been shown that there is a correlation between the development of other types of kidney stones and microbial ecological dysbiosis, in addition to infectious stones [115]. A small sample study comparing midstream urinary shotgun metagenomics in patients with calcium oxalate kidney stones and healthy subjects found substantial differences in microbial reads and overall genetic diversity of urinary tract microorganisms between patients with calcium oxalate stones and controls, suggesting that the development of calcium oxalate kidney stones may be associated with urinary tract flora closely associated with the urinary flora [140].

Many past studies have shown that engineered bacteria can play an important role in maintaining homeostasis of the human flora. They can maintain flora homeostasis by providing human microorganisms with probiotics, nutrients that promote microbial growth and function, suppressing ecological dysregulation caused by in vitro antibiotics, and competitively inhibiting pathogen colonization for the prevention and treatment of disease in a variety of ways. This suggests that regulating the balance of the urinary tract flora by altering the flora is one of the good and beneficial strategies [141–145]. Although research in this area is still in its infancy, studies have been conducted using E. coli 83972, extracted from the urinary tract of patients with asymptomatic infections, to competitively inhibit the colonization and growth of pathogenic bacteria [119]. Similarly, could better results be achieved using recombinantly engineered bacteria with more functionality and better efficacy? This does warrant further research.

The presence of microbial communities in the urinary tract allows for the engineered naturally occurring probiotics in the urinary tract to design probiotics with different functions for different types of kidney stones, either to inhibit the development of kidney stones by the directly degrading metabolites in the urinary tract or to regulate the homeostasis of the flora so that pathogenic bacteria do not colonise effectively. At the same time, the development of recombinant probiotics has faced several problems, such as being able to effectively colonise the urinary tract in a flushing environment, heterologous expression of proteins without causing damage to the urinary tract epithelium and inflammation and limiting pathogenic bacteria without causing damage to beneficial bacteria such as lactic acid bacteria. However, current research in this area is more limited to the engineering of the genome of urinary tract epithelial cells, and oxalate in the urinary tract can be effectively degraded by expressing oxalate-degrading genes in human embryonic kidney cells [146,147]. Although these studies of engineered human cells are difficult to actually apply in clinical practice, they do indirectly suggest that the idea of modifying the urinary tract flora to reduce the concentration of some metabolites in the urinary tract is feasible.

①Recombinant probiotics can degrade metabolites such as oxalate and uric acid in the intestine ②Recombinant probiotics can secrete a series of bioactive substances ③Bioactive substances can regulate flora homeostasis in the intestine and inhibit the onset of colitis ④Dysbiosis and colitis in the intestine promote the occurrence of kidney stones ⑤Recombinant probiotics prevent kidney stones by inhibiting the adhesion and growth of pathogenic bacteria ⑥Recombinant probiotics prevent kidney stones by regulating urinary tract flora homeostasis ⑦Oxalate, uric acid, pathogenic bacteria and dysbiosis will contribute to the occurrence of kidney stones.

4. Summary & Outlook

Calcium oxalate stones are the most common type of kidney stones, and high levels of oxalic acid in the urine increase the risk of developing these stones. As a result, there has been a significant effort to find ways to reduce urinary oxalic acid excretion in order to address this long-standing issue. However, supplementation with Oxalobacter formigenes and natural lactic acid bacteria has been shown to be ineffective in achieving this goal. Today, genetic engineering techniques are being used to modify the intestinal flora in order to acquire oxalate degradation capacity or to produce oxalate degrading enzymes. This breakthrough is important in addressing enterogenic hyperoxaluria as it allows for the degradation of oxalate in the intestine by probiotics.

There are four key enzymes now used for oxalate degradation: Frc and OxC, OxdC, OxO, whose common sources are Oxalobacter formigenes, Bacillus subtilis and Barley respectively. While certain bacteria have the ability to degrade oxalic acid, they are not commonly utilized for this purpose in the intestine due to limitations. There are now two main ways in which engineered microorganisms can be used to prevent and treat kidney stones: administration of recombinant bacteria and administration of genetically engineered enzymes, both of which have their advantages and disadvantages. Direct administration of heterologous enzymes may lead to a range of intestinal and immune reactions, but so far there have been no serious allergies to heterologous proteins in a series of animal and human studies with oxalate-degrading enzymes. However, this does not mean that these enzymes are safe and more research is needed to verify their safety. In the meantime, a recently developed bacterium capable of degrading uric acid in the intestinal tract may well avoid the series of infusion reactions and allergic

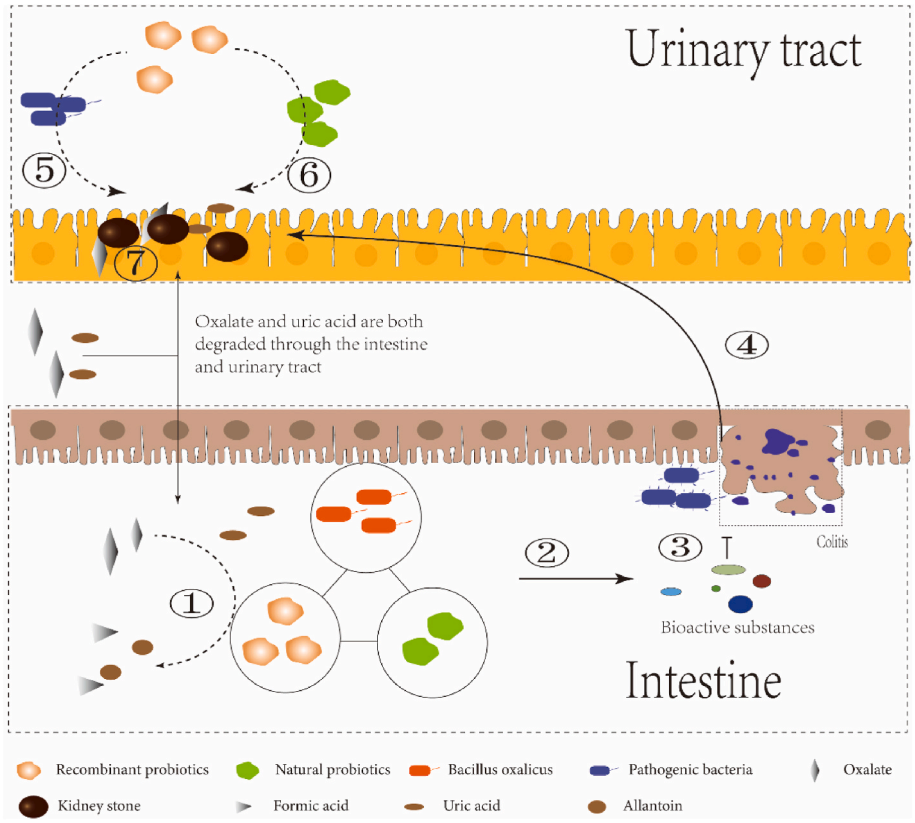

Fig. 2. Advances of the study of engineered microorganisms in kidney stone.

reactions associated with the injection of uric acid-degrading enzymes while achieving the effect of degrading uric acid in the intestinal tract, thereby reducing the renal uric acid load and inhibiting the development of uric acid stones, and is not expected to be absorbed into the body to cause a series of serious side effects.

A multi-species network of bacteria in the gut can work together to maintain oxalate homeostasis in healthy populations, and disturbances in the gut flora can mediate the production of kidney stones in a number of ways. The use of recombinant probiotics to produce a range of substances that are beneficial in maintaining gut flora homeostasis, such as SCFAs, is also an important breakthrough point in the prevention and treatment of kidney stones.

The urinary tract is not sterile; lactic acid bacteria and enterobacteria in the urinary tract are protective and promotive, respectively, against the development of kidney stones, and dysbiosis of the urinary tract flora will also lead to pathogenic bacteria and adhesion and kidney stones. The inherent probiotics in the urinary tract provide a good platform for modification to prevent the occurrence of kidney stones and can be engineered to be able to give these strains new functions to degrade metabolites and maintain the homeostasis of the urinary tract flora, but unfortunately, research in this area is still in the exploratory stage, and strains that are truly applicable to prevent the occurrence of kidney stones in the clinical setting have not yet been designed. However, if some natural microorganisms in the urinary tract are modified, it is highly likely that new advances will be made in the field of kidney stone prevention and treatment.

In summary, the gut and urinary tract microbiota are closely linked in the development of

various types of kidney stones. The accumulation of human metabolites in the kidneys can lead to the development of kidney stones, which can be prevented by modifying the gut and urinary

tract microbes to give them the ability to degrade these products (Fig. 2). At the same time,

homeostasis of the gut and urinary tract microflora is essential for maintaining a healthy renal

microenvironment, and any disruption of homeostasis will increase the risk of kidney stones.

Future research should aim to integrate engineered microorganisms more deeply into the gut and urinary tract flora to effectively reduce the concentration of metabolites in the urinary tract or inhibit the adherence of pathogenic bacteria without affecting or promoting flora homeostasis. In conclusion, engineered microorganisms can give new functions to intestinal and urinary tract

probiotics to prevent the development of kidney stones and has the potential to make new

advances in the prevention and treatment of kidney stones.

Author statement

Wenlong Wan: Writing original draft, Methodology, Data Curation, Investigation, Weisong Wu: Investigation, Data curation, Yirixiatijiang Amier and Yisheng Huang: Investigation, Data Curation, Xianmiao Li and Junyi Yang: Writing-Review & Editing, Xiao Yu and Yang Xun: Conceptualization, Writing - Review & Editing, All authors approved and contributed to the final manuscript.

Declaration of competing interest

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