

# Potentials and limitations of microorganisms as renal failure biotherapeutics

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**Abstract:** Renal insufficiency leads to uremia, a complicated syndrome. It thus becomes vital to reduce waste metabolites and regulate water and electrolytes in kidney failure. The most common treatment of this disease is either dialysis or transplantation. Although these treatments are very effective, they are extremely costly. Recently artificial cells, microencapsulated live bacterial cells, and other cells have been studied to manage renal failure metabolic wastes. The procedure for microencapsulation of biologically active material is well documented and offers many biomedical applications. Microencapsulated bacteria have been documented to efficiently remove urea and several uremic markers such as ammonia, creatinine, uric acid, phosphate, potassium, magnesium, sodium, and chloride. These bacteria also have further potential as biotherapeutic agents because they can be engineered to remove selected unwanted waste. This application has enormous potential for removal of waste metabolites and electrolytes in renal failure as well as other diseases such as liver failure, phenylketonuria, and Crohn's disease, to name a few. This paper discusses the various options available to date to manage renal failure metabolites and focuses on the potential of using encapsulated live cells as biotherapeutic agents to control renal failure waste metabolites and electrolytes.

**Keywords:** renal failure, microencapsulation, artificial cells, oral administration, bacterial cells, metabolites, electrolytes, polymeric membrane

## Renal failure, waste metabolites, and electrolytes

Kidney disease is among the most common diseases afflicting over 20 million Americans. Over 90,000 fatalities occur every year due to kidney diseases. Nearly 350,000 Americans suffer from end-stage renal disease (ESRD), the final stage in chronic renal failure. Each year, the number of patients with chronic kidney failure increases by an astounding 11%!

Kidney malfunction results from a reduction in glomerular filtration rate and causes an increase in concentration of waste metabolites that are measurable in blood.<sup>1</sup> For example, in human waste, metabolites such as blood urea nitrogen (BUN) increase from 15 mg/dl to 100–300 mg/dl, serum creatinine increase from 1 mg/dl to 10–25 mg/dl, and considerable amounts of uric acid are also known to accumulate.<sup>2–4</sup> More specifically, in uremia, the concentration of uremic toxins such as ammonia, urea, phenols, indoles, and guanidino compounds such as N-methyl guanidine (NMG) and guanidino succinic acid (GSA) are significantly altered with accompanying abnormalities in acid-base equilibrium, and retention of electrolytes and water retention.<sup>5,6</sup> The accumulation of uremic toxins in patients suffering from renal insufficiency inhibits various physiologic and biochemical functions thereby manifesting

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toxic symptoms. For example, hyperammonium can lead to mental retardation and, in severe cases, coma.<sup>7</sup> Water retention causes edema and as the concentration of hydrogen ions increases, acidosis develops. If untreated, acidosis and uremia can cause coma and eventually result in death. Thus, the high concentrations of these substances in have to be lowered before their increased level results in severe disturbances of metabolic pathways. Thus, as the kidney is accountable for the elimination of wastes from the blood, any damage, either from an accident or disease that causes renal insufficiency in the patient, can lead to a build-up of toxic waste in the body. Removing urea and ammonia from the plasma is not only necessary in kidney failure,<sup>1,8–11</sup> but also in other diseases like liver failure.<sup>1,8,12,13</sup>

## Treatment options for managing renal failure waste metabolites and electrolytes

Effective treatments for renal failure and elucidation of uremia have traditionally been dialysis or a kidney transplant. Only 15% of the world's uremic patients can afford dialysis treatment, as it is a very expensive, time-consuming, and complicated technique.<sup>4,14–16</sup> About 80,000 Americans on dialysis die of various complications each year. As a result of the global shortage of kidney donors for kidney transplants, high costs linked with transplant surgery, and high probability of organ rejection, most patients worldwide have very few options for effective treatment after kidney failure. Over 27,000 patients are on waiting lists for kidney transplants each year and only about 11,000 receive transplants. There is great urgency in the quest for an unconventional, affordable therapy for patients who cannot afford expensive dialysis or kidney transplant to keep them alive. Medical scientists are attempting to research and develop an innovative, low expense therapy that goes beyond the traditional treatment for reinstated kidney function. Several alternatives have been considered.<sup>4,9,10,17–23</sup>

In the past few decades, molecular technology has greatly influenced the course of biomedical research. Prospective medical applications include discovering the genetic basis for certain ailments, gene therapy, and production of effectual therapeutic agents. A distinguished researcher, Dr Kolff, found that creatinine, uric acid, and other nitrogenous wastes can easily be removed from plasma with oral sorbents.<sup>20</sup> With the use of oral absorbents, the interval between hemodialysis treatments could be delayed considerably.<sup>21</sup> Some researchers have proposed and confirmed the use of the co-immobilized

enzyme, urease, which breaks down urea into ammonia which is then eliminated by adsorbents.<sup>9,10,17,18</sup> However, some scientists report that the currently available ammonia adsorbent does not have adequate adsorbent capacity.<sup>16,19</sup>

An alternative approach has been suggested that would be cheaper and more convenient for the patient: an oral therapy containing a combination of adsorbents, osmotic agents, and ion-exchange agents.<sup>24</sup> Chang examined the potential of combining a microencapsulated enzyme, urease, with an ammonium ion adsorbent, zirconium phosphate, to remove urea *in vitro*. Urease broke down urea to ammonium ions which were then adsorbed onto zirconium phosphate. This system had the ability to delay the onset of dialysis therapy in patients with partial kidney function and may even decrease the treatment times for dialysis in some patients.<sup>25</sup> Oxystarch and urease–zirconium phosphate have been demonstrated to be successful in removing urea and ammonia.<sup>2,4</sup> However, the amount required is too large to allow for use in routine treatment of the patients.<sup>26</sup> and also markedly small amount of urea removal have been reported *in vivo*, particularly at a neutral pH.<sup>6,13,15,19,23,27–30</sup>

In another approach, a microencapsulated multienzyme system that converts urea and ammonia into essential amino acids was investigated.<sup>13,18,19,28</sup> However, this encapsulated multienzyme system had an inadequate urea and ammonia conversion rate.<sup>31</sup> Thus, the desire for an efficient system for urea and ammonia removal is apparent.<sup>32</sup> It is essential to develop novel approaches to replace kidney function that majority of the world can afford. One prospect involves creation of a 'bioartificial/bionic kidney' in which normal renal function are performed by tissue culture cells implanted in a hollow fiber or mesh matrix.<sup>33</sup> In spite of great potential for the bionic kidney, it, too, will be exorbitantly expensive and will require extremely skilled personnel. In a recent article in the April 2007 issue of *The FASEB Journal*, Cody Mooneyhan described the use of puffer fish gills to excrete ammonia at the molecular level using Rh proteins and reported that the protein which excretes ammonia through puffer fish gills was found to be similar to human Rh blood proteins. Thus, by targeting human Rh proteins, people with damaged livers and kidneys can eliminate toxic ammonia from their bloodstream.<sup>34</sup>

Stem cells are unspecialized precursor cells that can self-renew and develop into specialized cells. Stem cell researchers are working towards the ultimate goal of production of a new kidney as a mean of kidney therapy. This is a complicated challenge since the kidney is made from several specialized cell types, each with its own

unique function. Another strand of biotechnology that holds promise in the treatment of kidney failure is the use of bioencapsulated living cells.<sup>35</sup> It is possible to genetically engineer nonpathogenic bacterial cells for a desired metabolic activity and they then serve as outstanding therapeutic agents. The idea of administering encapsulated bacterial enzymes to break down toxic substances was first introduced in Finland in 1978.<sup>32,36</sup> In one proposed system, selected bacteria converted nitrogenous waste products into nontoxic chemicals that could be recycled within the subject.<sup>24,37</sup> Thus, several approaches to treat renal insufficiency have been proposed.<sup>4,13,15,16,23,29,30,38–40</sup> Some researchers suggest the use of microencapsulated urease to convert urea into ammonia that is subsequently removed by coencapsulated ammonia adsorbent<sup>9,14</sup> or as mentioned above, some researchers propose administering microencapsulated multienzyme complex to convert urea and ammonia into essential amino acids<sup>9,15,39,28</sup> or using lyophilized urea-utilizing soil bacteria.<sup>41,42</sup>

Microencapsulated cells have also been reported to be successful in other medical complications. For example, the microencapsulated islet cells were found to remain viable and secreted insulin to regulate glucose levels in diabetic rats. This approach prevented immunorejection after implantation.<sup>43</sup> Similarly, microencapsulated hepatocytes have been shown to lower the high serum bilirubin level to  $6.00 \pm 1.00$  mg/100 mL twenty days after implantation in Gunn rats. Analysis showed that this was achieved because the implanted encapsulated hepatocytes carried out the function of the liver in the conjugation of bilirubin<sup>44,45</sup> and enhanced the survival time of fulminant hepatic failure in rats.<sup>45</sup> Recent reports by another group also support this finding.<sup>46</sup> The possibility of using live selected bacteria, preferably *Bacillus pasteurii* or *Lactobacillus sporogenes*, *in vivo* to treat renal, hepatic and gastrointestinal diseases by eliminating toxins and other metabolic waste products has been proposed.<sup>7</sup> The study also showed that a probiotic containing either *B. pasteurii* and *L. sporogenes*, or both, are capable of increasing survival in otherwise untreated uremic rats.

In another approach to alleviate uremia or renal insufficiency, a mixture comprising of one or more selected bacteria (which converts nitrogenous waste into nontoxic compounds *in vivo*), along with one or more of the following: a prebiotic, ammoniophilic bacteria with high urease activity, and/or sorbents with specific adsorption affinities for uremic toxins and inorganic phosphate along with a water sorbent have been proposed.<sup>43</sup> A series of probiotics (foods that

contain 'beneficial' bacteria, such as *Lactobacillus*), along with oral adsorbents like charcoal and locust bean gum, have also been explored and tested in subjects as a potential oral renal replacement therapy.<sup>47</sup>

There have been rapid advances in molecular biology that have resulted in the use of genetically engineered microorganisms for remedial purposes. Genetically engineered cells that were incapable of surviving passage through the gastrointestinal tract have made it successfully to their destination by the use of artificial cell microcapsules. Microorganisms can be engineered to remove unwanted molecules from the body as they travel through the intestine and are finally excreted in the stool without being retained in the body. This idea has great prospects and it is likely that soon trained bacteria will act as a substitute for the kidney and liver and perform most endocrine functions.

Microencapsulated genetically modified cells have been reported to have enormous potential for the elimination of certain metabolites such as urea in kidney failure, ammonia in liver failure, and amino acids such as phenylalanine in phenylketonuria and other innate errors of metabolism.<sup>48</sup> In addition, genetically engineered encapsulated *Erwinia herbicola* cells have demonstrated an ability to convert ammonia into usable amino acids for the cells before being eliminated via the bowel. Microencapsulated genetically engineered *Escherichia coli* DH5 cells have also been shown to be effective in removal of urea and ammonia in an *in vitro* system and in a uremic rat animal model.<sup>49,50</sup> Despite the research in this field, we are still looking for a suitable urea and ammonia removal system. The most promising approach, using microencapsulated bacterial cells for renal therapy, is discussed extensively in this article.

## Potential of live free and encapsulated cells in renal failure

About forty years ago, Malchesky first suggested that certain natural strains of microorganisms were exceptionally successful in degrading urea *in vitro*. He also reported that these microbes can be trained to enhance their ability to degrade urea and other compounds normally excreted in urine.<sup>51</sup> Soon after, Setala pioneered the notion of oral delivery of lyophilized bacteria harvested from soil.<sup>36</sup> These bacteria were extremely effective in degrading nonprotein nitrogenous compounds in uremic patients.

Later, in the 1990s, Chang established the concept of delivery of microencapsulated genetically modified bacteria to

degrade nitrogenous waste products in uremic patients *in vivo* as well as *in vitro*. The procedures for microencapsulation of biologically active materials are well recognized and offer various biomedical applications.<sup>9,11,13,17,22,31,52–54</sup> Thus, the possibility of using bacterial cells to treat kidney failure has been explored for over four decades. Research in the field of artificial cell microcapsules revealed the possibility of oral administration of live genetically engineered cells for therapeutic functions.<sup>32,55</sup> This concept has direct relevance for the use of encapsulated bacterial oral therapy in renal failure and liver failure,<sup>32</sup> physiologically responsive gene therapy,<sup>56,57</sup> and somatic gene therapy.<sup>58,59</sup> Modern technological advances in molecular biology have resulted in the accessibility of nonpathogenic genetically engineered microorganisms that can effectively use uremic metabolites for cell growth. This paper is an overview of the options available to overcome renal sufficiency. One of the options discussed extensively is the current research on using microencapsulated bacterial cells such as *E. coli* DH5 to degrade waste metabolites such as urea, uric acid, and creatinine among others, as an improved therapy of renal failure. The results obtained upon oral administration of microencapsulated bacterial cells to degrade such waste metabolites in uremia are summarized here.

## Potential of encapsulated cells in renal failure urea removal

Renal insufficiency results in an elevated plasma urea level. Several approaches have been suggested to degrade plasma urea. In the 1980s, the novel approach of using encapsulated bacteria was shown to be 10 times more efficient in degrading urea than oxystarch. One gram of oxystarch was found to adsorb only 103.00 mg of urea at pH 7.4 at a urea concentration of 0.02 M.<sup>5</sup> Thus, to eliminate 40 g of urea from 40 L fluid (100 mg/dL urea), 388.34 g of oxystarch was required. Microencapsulated genetically engineered bacteria were reported to be 30 times more efficient compared to microencapsulated enzyme urease–zirconium phosphate. The encapsulated urease–zirconium-phosphate system only eliminated 1.60 mg of urea nitrogen or 33.00 mg urea/g of microcapsules.<sup>5</sup> Therefore, massive quantities of microcapsules containing this system were needed to successfully remove 40 g of urea from the body.

Certain bacterial cells are reported to be very effective in lowering BUN levels *in vivo*. When partially nephrectomized rats were orally given *B. pasteurii* and *L. sporogenes*, the BUN levels were lowered to  $62.0 \pm 21$  and

$63.0 \pm 26$  mg/dl, respectively compared to a previous concentration of  $99.0 \pm 46$  mg/dl. This reduction of 38% and 37%, respectively indicated that *B. pasteurii* and *L. sporogenes* administered orally as dietary supplements could metabolize urea *in vitro*.<sup>7</sup>

Alternatively, the use of probiotics in removal of plasma urea has also been explored. Suspension of *L. delbrueckii* in uremic plasma reduced the urea nitrogen levels from  $51.5 \pm 5.2$  mg/dL to  $44.3 \pm 3.9$  mg/dL ( $p = 0.02$ ) after 24 hours *in vitro*. With microencapsulation of *Lactobacillus* (inside semipermeable alginate–polylysine–alginate polymeric membrane [APA]), further lowering of urea nitrogen levels was achieved ( $35.4 \pm 0.8$  mg/dL,  $p = 0.03$ ) at 24 hours. It is proposed that expression of certain enzymes could be induced in *L. delbrueckii* which can then effectively lower plasma urea<sup>60</sup> and possibly other waste metabolites in uremia.

Recently, Chang and Prakash proposed the use of microencapsulated genetically engineered bacterial cells to remove plasma urea and ammonia. *In vitro*,  $40.00 \pm 8.60$  g of APA-encapsulated bacteria were shown to remove  $87.89 \pm 2.25\%$  of the plasma urea within 20 minutes and 99.99% of urea in 30 minutes. Bacterial cells were reported to use urea for their metabolic nitrogen requirement and did not produce ammonia as a by-product.<sup>57</sup> Thus, results demonstrate that this biotechnological approach is 10–30 times more competent in eliminating urea and ammonia than the currently available traditional approaches.<sup>31</sup>

In a different study, surgical renal failure induced in rats (removal of one kidney and the partial ligation of the other) resulted in a substantial increase in blood urea levels without noticeable disturbances in water and electrolyte balances. A drop in the plasma urea level from  $52.08\% \pm 2.06\%$  mg to  $9.10\% \pm 0.71\%$  mg was observed upon daily oral administration of log phase microencapsulated genetically engineered *E. coli* DH5 cells for 21 days. The plasma urea level was maintained within the normal range during the entire treatment period. The urea levels became elevated once the treatment was stopped.<sup>61,62</sup> It is hypothesized that during the passage of microcapsules through the gastrointestinal tract, small molecules from the body, such as urea, ammonia, amino acids, etc., diffuse into the microcapsules where they are metabolized by genetically engineered cells for their nitrogen source before being excreted in the stool. This results in lowering the high plasma urea level to standard levels in uremic rats with induced kidney failure.<sup>32</sup> Since urea levels returned to pretreatment values upon stopping the treatment, it is implied that there is no significant retention of *E. coli* DH5 cells in the intestine.<sup>63</sup>

One can argue about the potential toxicity due to the leakage of encapsulated cells during their passage through the intestinal tract. In the *in vivo* studies, the oral administration of both free and encapsulated bacteria to uremic rats lowered systemic urea from the initial 52.08 (SD = 2.37) mg/dl to 10.58 (SD = 0.85) mg/dl. However, the free *E. coli* DH5 cells were found to be retained in the intestine and when the treatment ceased, the free bacteria were still able to lower systemic urea.<sup>62</sup> Unlike free bacteria, microencapsulated bacteria were excreted in the stool. The results showed that, even if all the *E. coli* DH5 cells leaked out, there would be no negative effect on the growth and/or survival of the renal failure rats.<sup>64</sup> It is reported that uremic rats survived longer when they received encapsulated *E. coli* DH5 cells.<sup>64,65</sup>

### Potential of encapsulated cells in renal failure ammonia removal

The fate of ammonia during urea removal by daily administration of microencapsulated genetically engineered bacteria has also been examined. The blood ammonia levels that were always present in the range of  $539 \pm 51 \mu\text{M}$  decreased significantly to  $144 \pm 24.70 \mu\text{M}$ .<sup>32</sup> *In vitro*,  $40.00 \pm 8.60 \text{ g}$  of APA-encapsulated bacteria have been reported to lower ammonia from  $975.14 \pm 70.15 \mu\text{M/L}$  to  $81.151 \pm 7.37 \mu\text{M/L}$  in 30 min.<sup>31</sup> The most recent studies in uremic rats also showed a reduction of other waste metabolites such as, uric acid, creatinine, potassium, and phosphate.<sup>55,66,67</sup>

### Potential of encapsulated cells in renal failure creatinine removal

High level of plasma creatinine occurs in renal insufficiency, uremia, and other diseases. Serum creatinine levels were reported to be lowered in rats fed with *B. pasteurii* and *L. sporogenes* from  $0.9 \pm 0.25 \text{ mg/dl}$  and  $0.9 \pm 0.2 \text{ mg/dl}$ , respectively compared to a previous concentration of  $1.5 \pm 0.56 \text{ mg/dl}$ . A substantial reduction of approximately 40% in both groups was observed. These results indicate that *B. pasteurii* and *L. sporogenes* administered orally as dietary supplements metabolize creatinine *in vitro*.<sup>43</sup>

Daily administration of microencapsulated genetically engineered *E. coli* DH5 cells have resulted in lowering plasma creatinine *in vitro* and *in vivo*. Results demonstrated that these artificial cells were able to lower plasma creatinine *in vitro* from  $21.80 \pm 1.10 \text{ mg/dl}$  to  $19.34 \pm 0.60 \text{ mg/dl}$  in three hours.<sup>67</sup> It would be interesting to see if the creatinine levels were elevated again upon cessation of the oral treatment.

### Potential of encapsulated cells in renal failure uric acid removal

Increase in systemic uric acid occurs in renal insufficiency. Recently, a proposal was put forth to use artificial cells containing microencapsulated genetically engineered *E. coli* DH5 cells for lowering uric acid *in vitro* and *in vivo*. Results show that genetically engineered bacteria have the ability to significantly lower uric acid from  $84.80 \pm 3.40 \text{ mg/dl}$  to  $9.32 \pm 0.05 \text{ mg/dl}$  *in vitro*. They were also capable of lowering uric acid levels from the plasma of the experimental animals from the control levels of  $71.00 \pm 27.49 \text{ mg/dl}$  to  $20.33 \pm 17.92 \text{ mg/dl}$  *in vivo*.<sup>55</sup> Continued daily oral administration reduced the plasma uric acid concentration to normal range in uremic rats during the entire test period. Its potential in the removal of uric acid may have significance in uremia.

### Potential of encapsulated cells in removal of other renal failure waste metabolites

Lowering of plasma magnesium, phosphate, sodium, chloride, uric acid, cholesterol, and creatinine is essential in uremia and other diseases.<sup>5,68</sup> Microencapsulated genetically engineered microorganisms have been prepared that can remove waste metabolites such as potassium, phosphate, magnesium, sodium, chloride, uric acids, cholesterol, creatinine, and bilirubin *in vitro*. This has a significant implication in the use of oral microencapsulated genetically engineered microorganisms in uremia. These artificial cells were effective in removing the majority of waste metabolites from the plasma as summarized in Table 1. Further studies will reveal whether administration of the microencapsulated *E. coli* cells will cause a similar reduction of the above mentioned electrolytes and waste metabolites *in vivo*. This has exciting implications for the use of genetically engineered cells in a number of medical applications. However, encapsulated *E. coli* DH5 cells could not efficiently remove creatinine from the plasma. After 24 hours of incubation with encapsulated bacteria,  $83.31\% \pm 2.40\%$  plasma creatinine was found remaining.<sup>69</sup>

All of the uremic metabolites tested, urea, cholesterol, uric acid, potassium, phosphate, magnesium, chloride, sodium and to a certain extent creatinine, are lowered to within normal levels without elevation of ammonia. The effectiveness of this approach was explored by studying the survival and growth of the renal failure rats receiving microcapsules containing genetically engineered *E. coli* DH5 cells. In this respect, untreated uremic control animals

**Table I** Removal of electrolytes by microencapsulated genetically engineered *E. coli* DH5 cells *in vitro*<sup>69</sup>

Metabolites	Concentration at 0 hours	Concentration at 24 hours
Potassium	5.80 ± 0.40 mEq/l	3.50 ± 0.03 mEq/l (p < 0.001)
Phosphate	2.20 ± 0.9 mg/dl	1.49 ± 0.03 mg/dl (p < 0.005)
Magnesium	0.90 ± 0.04 mg/dl	0.66 ± 0.09 mg/dl (p < 0.005)
Sodium	172 ± 11.00 mEq/l	129 ± 6.12 mEq/l (p < 0.001)
Chloride	137 ± 6.60 mEq/l	107 ± 2.00 mEq/l (p < 0.005)
Uric acid	84.80 ± 3.40 mg/dl	8.80 ± 3.12 mg/dl (p < 0.001)
Cholesterol	1.86 ± 0.10 mmol/l	1.37 ± 0.06 mmol/l (p < 0.005)

died during the study period of 21 days, whereas the treated animal continued to survive and grew at about the same rate as normal animals.<sup>64</sup> Another alternative to the oral administration of microencapsulated genetically engineered cells has been proposed by some researchers: the long-term implantation of cells. This can be an effective therapy for various conditions but will take numerous years to perfect. In the meantime, several researchers are looking into other approaches for a more immediate clinical application. For example, Aebischer's group suggested the ingenious use of capillary fibers to encapsulate cells that has permitted his group to insert these subcutaneously into the cerebrospinal fluid on a short-term basis.<sup>70</sup> The capsules can be replaced as needed,<sup>70</sup> and, so far, no immunological sensitivity to the capsules is obvious. This approach evades the problems posed by permanent or long-term implantation of microcapsules. The oral administration of microencapsulated genetically engineered cells obviates the problems of implantation. However, it can be used only for diseases where waste metabolites can be eliminated from the gastrointestinal tract, such as in liver or kidney failure and in some innate errors of metabolism such as phenylketonuria.<sup>48</sup> Evidently, the clinical use of microencapsulated genetically engineered cells could require a combination of different approaches, including implantation, subcutaneous insertion, and oral administration, to treat different conditions.<sup>63</sup>

Immobilized bacteria have demonstrated an unlimited capacity to deplete cholesterol levels *in vitro*.<sup>57</sup> However, for practical applications, suitable bacteria with an enhanced ability to degrade cholesterol are desirable. There is a strong possibility that this method may become accessible in the near future with the help of genetic engineering. Research in other systems such as using bioencapsulated hepatocytes has shown promising results and demonstrated the feasibility of using this for cell therapy.<sup>57</sup> Further enhancement in biocompatibility may allocate this approach to be used for cell and gene therapy in humans. This is becoming a

progressively more viable prospect because of the increasing advancement in genetic engineering and molecular biology. There has been an extensive growth in technical research in molecular biology leading to the generation of many genetically engineered microorganisms with unique and exceptional abilities. Microorganisms have been easily manipulated to overproduce enzymes and peptides and some have been engineered to metabolize large amounts of unwanted metabolites.

Recently, Prakash and Chang<sup>31,32,71</sup> have reported oral administration of microencapsulated genetically engineered *E. coli* cells containing the *Klebsiella aerogenes* urease gene that causes the overexpression of the urease enzyme efficiently removes urea from the reaction media. Microencapsulated genetically engineered *E. coli* cells have the competence of efficiently removing urea without any increase in the ammonia levels in the medium.<sup>69</sup> Using a single pool model, 40 g of microencapsulated genetically engineered *E. coli* cells could lower urea in 40 liters of the body water from 100 mg/dl to 1.60 mg/dl within 30 minutes. Also, 40 g of this microorganism was shown to lower ammonia in 40 liters of body water from 758.00 µM/l to 90.42 µM/l in as little as 20 minutes. Extrapolated results imply that the ability of microencapsulated bacteria to degrade urea is adequate to eliminate urea during renal insufficiency.<sup>72</sup> Analogous reductions in blood levels of other metabolites have also been reported, implying that the DH5 cells have the capacity to regularize levels of several elevated metabolites during renal failure.<sup>32,58,73</sup> Detailed studies have been done on the optimization of procedural parameters for encapsulation of bacterial cells in the APA membrane and the profiles of genetically engineered microencapsulated bacteria to effectively remove urea and ammonia and the efficacy of urea and ammonia removal *in vitro*.<sup>31</sup> In addition to its potential use in uremia, the removal of waste metabolites such as urea, ammonia, uric acid, bilirubin, and others are also required in other medical conditions such as liver failure.<sup>43</sup>

**Table 2** Potential of using cells as a mode of therapy for renal failure and other diseases

Renal failure markers and disease conditions	Cell types	Potential mode of therapy	Reference
Urea in renal failure, amyotrophic lateral sclerosis	Free live <i>E. coli</i> DH5	Oral	58, 62
	Microencapsulated genetically engineered bacteria	Oral	31, 49, 57, 63, 66, 71, 73, 74
	Microencapsulated <i>E. coli</i> DH5	Oral	62
	<i>E. coli</i> with <i>Klebsiella aerogenes</i> expressing urease	Oral	72
	Soil bacteria	Oral	41, 42
	Encapsulated genetically modified xenogenic cells	Intrathecal delivery	70
	Selected probiotic ( <i>B. pasteurii</i> , <i>L. sporogenes</i> ), ammoniaphilic bacteria	Oral	7, 43, 50
	Probiotic microencapsulated <i>L. delbrueckii</i>	Oral	50, 65
	Microencapsulated multienzyme complex	Oral	9, 15, 28, 39
Ammonia in renal failure and liver disease	Microencapsulated multienzyme complex	Oral	9, 15, 28, 39
	Soil bacteria	Oral	41, 42
	Microencapsulated genetically engineered bacteria	Oral	31, 32, 43, 49, 50, 57
	Microencapsulated <i>E. coli</i> DH5	Oral	57, 71
Uric acid in renal failure	Microencapsulated genetically engineered bacteria	Oral	43, 66, 69, 74
	Microencapsulated <i>E. coli</i> DH5	Oral	55, 66, 67
Creatinine in renal failure and other diseases	Microencapsulated genetically engineered bacteria	Oral	66, 69
	<i>L. sporogenes</i> , <i>B. pasteurii</i>	Oral	43
	Microencapsulated <i>E. coli</i> DH5	Oral	55, 66, 67
Other metabolites; potassium/phosphate/magnesium/sodium/chloride in uremia and other diseases	Microencapsulated genetically engineered microorganisms	Oral	69
Nonprotein nitrogenous compounds in uremia	Lyophilized soil bacteria	Oral	41, 42
Insulin in diabetes	Microencapsulated islet cells	Implantation	43
Serum bilirubin in fulminant hepatic failure rats	Encapsulated hepatocytes	Implantation	44–46
	Microencapsulated genetically engineered microorganisms	Oral administration	43, 55, 66, 67, 69
Toxins and other metabolic wastes in renal, hepatic and gastrointestinal diseases	Live selected bacteria: <i>B. pasteurii</i> and <i>L. sporogenes</i>	Oral administration	7

O'Loughlin and colleagues have recently undertaken a study to demonstrate the competence of a combination of genetically engineered bacteria to lower elevated levels of metabolites such as urea and uric acid. Two strains of genetically modified bacteria, one expressing enzyme 'urease' to degrade urea and the other expressing enzyme 'uricase' to degrade uric acid, were prepared, combined and encapsulated in APA microcapsules for oral administration. Within 24 hours, 5 mL of these capsules were capable of

successfully eliminating 95% of the urea and over 99% of the uric acid *in vitro* from a 100 mL solution formulated to mimic the concentration of these solutes in a hemodialysis patient.<sup>74</sup> This approach could potentially serve in conjunction to maintenance dialysis in patients with chronic renal failure. However, reduction of urea concentration *in vivo* required coadministration of an ion-exchange resin to adsorb ammonia. Reduction of uric acid concentration *in vivo* was very efficient and did not require the administering

of ion-exchange resin. Oral delivery of a combination of genetically engineered bacterial cells should be further investigated as a valuable accessory to dialysis and/or to immunosorption for the treatment of uremia<sup>74</sup> in chronic kidney failure models.

O'Loughlin and colleagues pioneered the approach of using a combination of enzymes in a single delivery vehicle to degrade multiple uremic toxins in a nonbacterial system. An alginate microcapsule was developed that contained three enzymes, urease, uricase, and creatininase, which are capable of effectively degrading urea, uric acid, and creatinine, respectively, and are significantly elevated in patients with renal insufficiency. The microcapsules were evaluated both *in vitro* and *in vivo* in a rodent model. *In vitro*, 5 mL of the capsules equipped with a few milligrams of the enzymes within 24 hours effectively degraded 100% of the uric acid, 97% of the urea, and 70% of the creatinine in a 100 mL formulated solution that mimicked the concentration of these solutes in uremic plasma. *In vivo* experiments involved a chemically induced acute renal failure rat model to evaluate the ability of encapsulated enzymes along with an oral sorbent (ion-exchange resin) to degrade uremic toxins.<sup>75</sup> This approach has the potential to be extended to a bacterial system and has considerable prospect of being to be used in conjunction therapy in the treatment of ESRD.

## Oral administration of encapsulated cells live cells as potential renal failure kidney substitute

It has recently been demonstrated that daily oral administration of artificial cells microcapsule containing a genetically engineered microorganism has potential as a renal failure kidney substitute. Furthermore, these genetically modified bacteria also have the ability to remove potassium, phosphate, uric acid, and other waste metabolites from uremic plasma. Future research may lead to the efficient and effective use of microencapsulated genetically modified bacteria as therapeutic agents.<sup>58</sup>

The accessibility of an oral bacterial therapy has the potential to save over 400,000 lives worldwide each year. Research has demonstrated that these microencapsulated genetically engineered bacteria are much more successful in removing ammonia than any method currently available.<sup>31,32,75</sup> If further detailed investigation convinces us about the efficacy and safety of this approach for urea removal, it might be possible to use this technology to remove waste metabolites such as urea, ammonia, creatinine, and uric acid

in uremic patients with chronic renal failure. Because of these encouraging results, further research will concentrate on experimental design to use the concept of oral administration of genetically engineered bacterial cells in combination with other technologies for *in vivo* use. Thus, this approach will complete the currently available oral therapy for uremia using adsorbents, ion-exchange resins, and osmotic agents. The oral administration approach might therefore also be applicable for the removal of ammonia in other diseases such as chronic liver failure.

The use of microencapsulated bacterial cells has potential application not only in renal insufficiency, innate errors of urea metabolism, but also in liver insufficiency and gastrointestinal disorders and diseases.<sup>43</sup> The feasibility of oral administration of polymeric artificial cells containing genetically engineered cells for the specific removal of undesirable amino acids in some innate errors of metabolism as in phenylketonurea<sup>48</sup> has been reported. Further detailed studies on efficacy and safety are required before this promising new approach can be fully recognized and applied.

Microencapsulated genetically modified cells can be administered orally to a subject with uremia to alleviate the symptoms of uremia. Depending on the extent of renal damage in the patient suffering from uremia, with this oral therapy the patient either will not require dialysis, require dialysis less frequently, and for shorter periods, or maybe will not require initiation of dialysis as early as would be needed without treatment. In initial stages of ESRD, before fluid retention, genetically modified bacterial cells can be administered orally alone. However, in later stages with fluid retention in patients, the oral approach could be combined with minute quantities of an oral osmotic agent such as mannitol,<sup>24</sup> to remove about a liter of fluid per day. Recent demonstrations have implied that daily dialysis avoids large fluctuations in the systemic waste metabolites. This oral approach could be used as in combination with standard dialysis to prevent large fluctuations in the systemic waste metabolites. The swift and proficient removal of uric acid may have added potential applications in hyperuricemia, such as in gout and in chemotherapy.<sup>66</sup> Future studies will unveil the potential use of encapsulated genetically engineered bacteria for removal of urea and ammonia in biotechnology, chemical engineering, and biomedical applications.<sup>31</sup>

Will a combination of microencapsulated *E. coli* DH5 cells to deplete urea, and oral adsorbents and osmotic agents to regulate water, electrolytes, and other uremic waste metabolites remove the need for dialysis entirely in patients with kidney failure?<sup>63</sup> Will the oral administration of these

genetically modified bacterial cells to uremic patients result in decreased frequency of dialysis or perhaps even decreased dialysis treatment times?<sup>51</sup> Further research could conceivably produce an alternative treatment of chronic renal failure and provide an insight for the future direction of this emergent and highly prospective technology. This also has potential applications in cell and gene therapy.<sup>57</sup>

## Conclusion

Kidney failure leads to uremia, a complicated syndrome associated with increased levels of unwanted metabolites and electrolytes.<sup>3–5</sup> It becomes essential to remove waste metabolites and regulate water and electrolytes in patients with renal insufficiency. Standard treatment by dialysis or transplantation is very effective but is extremely expensive and unaffordable by the majority of the world. Researchers of other approaches including absorbents, are faced with one major challenge, the need to remove large amounts of urea, ammonia, and other waste metabolites and electrolytes.<sup>4–6,16,19,22,23,28,76</sup> Currently, artificial cells are being investigated with great optimism for use in the replacement of cell and even organ functions, especially related to metabolic functions in the treatment of diseases such as diabetes, liver failure, and kidney failure. When artificial microencapsulated cells are given by implantation,<sup>13,36,77–80</sup> The problem of retention in the body is eliminated due to the use of polymeric membranes. Orally administered microencapsulated live cells warrants investigation as a supplement to the routine dialysis system to avert large fluctuations in the systemic waste metabolites and electrolytes. Further detailed analysis, particularly on safety studies are vital. If proven safe, this approach of using microencapsulated live cells will be an economical means for managing renal failure redundant waste metabolites and electrolytes.

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