THERAPEUTIC APPLICATIONS OF POLYMERIC ARTIFICIAL CELLS

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Abstract | Polymeric artificial cells have the potential to be used for a wide variety of therapeutic applications, such as the encapsulation of transplanted islet cells to treat diabetic patients. Recent advances in biotechnology, molecular biology, nanotechnology and polymer chemistry are now opening up further exciting possibilities in this field. However, it is also recognized that there are several key obstacles to overcome in bringing such approaches into routine clinical use. This review describes the historical development and principles behind polymeric artificial cells, the present state of the art in their therapeutic application, and the promises and challenges for the future.

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doi:10.1038/nrd1659

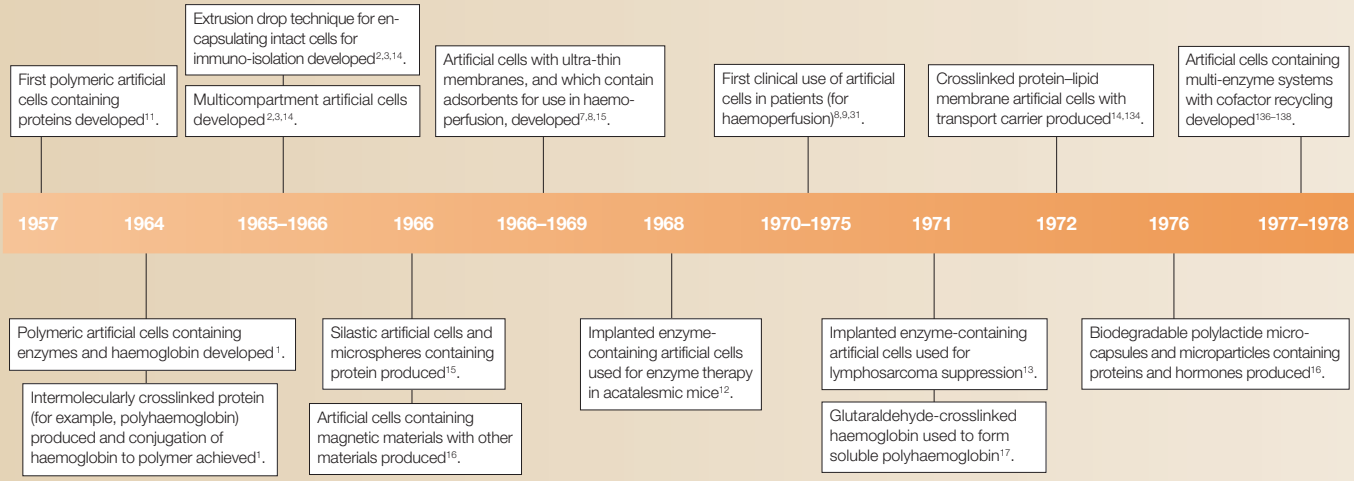
The concept of using 'artificial cells' — ultra-thin polymer membrane microcapsules — to encapsulate materials such as transplanted cells, enzymes and absorbents was first put forward 40 years ago[1–3] (TIMELINE). Encapsulation was proposed as a means to protect the enclosed materials from the external environment, thereby helping to prevent rejection by the immune system[1–3]. Since then, significant advances have made the translation of this concept to clinical use — for example, in the treatment of type 1 diabetes using encapsulated islets[4] — increasingly practicable[5,6]. However, there are a number of key challenges that need to be addressed before products based on these approaches can enter clinical development[6], which will be discussed in this article.

The first routine clinical application of artificial cells — in this case, those encapsulating adsorbents such as activated charcoal — has been in removing toxins and drugs from the blood[7–10]. Numerous other applications of polymeric artificial cells have emerged, because many types of biologically active materials, such as enzymes, can be micro-encapsulated individually or in combination[1–3,11–20] (FIG. 1), as well as whole cells (FIG. 2).

Biologically active materials inside the artificial cells are prevented from coming into direct contact with external entities such as leukocytes, antibodies or tryptic enzymes (FIG. 1). However, smaller molecules, such as hormones, peptides and small proteins, can equilibrate rapidly across the membrane of artificial cells. The ultra-thin membrane (0.02 μm) and membrane equivalent pore radius of 14 Å, together with the large total surface area of the artificial cells (10 ml of 20-μm-diameter artificial cells have a total surface area of about 20,000 cm^2 — equivalent to that of a haemodialysis machine), allows for extremely fast equilibration of molecules smaller than large proteins.

The permeability, composition and configuration of the membrane can be varied using different types of materials, which allows for extensive variations in the properties and functions of artificial cells. The artificial cells can also range in size from macro-dimensions of up to 2-mm diameter, through micron- and nano-dimensions down to molecular dimensions (BOX 1). Now that drug discovery based on biotechnology and molecular biology has come of age, the potential therapeutic applications of polymeric artificial cells are just beginning to be realized and demonstrated. The discussion that follows focuses on polymeric artificial cells, including the principles, the development, present status, future perspectives and challenges. This review does not discuss the use of polymeric artificial cells in drug delivery systems, as excellent reviews of this field have already been published in this journal[21–23].

Timeline | **Concepts and applications of polymeric artificial cells**



Artificial cells containing bioadsorbent

As mentioned above, the microscopic dimensions of artificial cells result in a large surface-to-volume ratio. This, together with the ultra-thin membranes, makes artificial cells that contain bioadsorbents much more effective in removing toxins and drugs from the blood of patients when compared with standard haemodialysis[7–10]. The most common and earliest such approach is the use of microscopic polymeric artificial cells that encapsulated activated charcoal[7–9,15], which solved the major problems relating to the release of embolizing particles and subsequent damage to blood cells when bioadsorbents were used without the artificial cell membranes. Since the early 1980s, this approach has been used routinely worldwide for the treatment of acute poisoning in adults and children, especially in suicidal overdose[9,10,24–30]. This is particularly useful in situations in which haemodialysis units are not easily accessible or available.

This approach has also been shown to effectively remove toxic uraemia products, resulting in the relief of uraemic symptoms in uraemic patients[8–10,14], although components for the removal of other uraemic metabolites need to be developed. In addition, such an approach can also remove toxic molecules in patients with liver failure, which has resulted in the recovery of consciousness in grade 4 hepatic coma patients[31,32]. Of course, detoxification is only one of the functions of the liver, but this approach is being used as the detoxification component of hybrid liver support systems that are being developed[33]. The success in the treatment of patients using artificial cells containing bioadsorbents for detoxification has led to increasing interest in research and development in many other areas, which are described below.

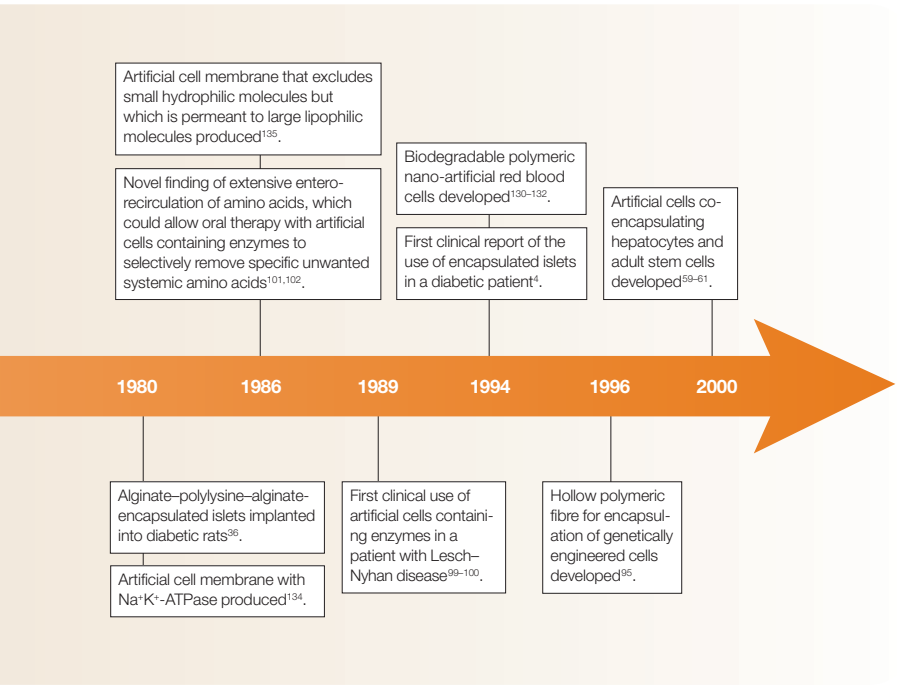
Artificial cells for cell encapsulation

Many attempts have been made to prevent the rejection of transplanted cells by the immune system by using dialysers, ultrafiltrators, membrane sacs, membrane disks and polymeric devices. However, in these configurations, one of the major problems is low cell viability, owing to limitations of mass transfer for oxygen and nutrients. Polymeric artificial cells with microscopic dimensions (less than 2 mm in diameter) provide a high surface-to-volume ratio, which allows for good mass transfer of oxygen and nutrients, while also acting to protect the cells from immunorejection[1–3,14].

There has been an increasing realization of the potential of cell encapsulation with polymeric artificial cells in recent years[6,34] (FIG. 2) — as highlighted, for example, by a 2003 consensus paper from several leading groups published in *Nature Medicine*[6]. Indeed, cell-based products, including cells with synthetic biomaterials, are now recognized as a new category of therapeutic products by the US Pharmacopeia and National Formulation[35].

Encapsulation of islets. As mentioned above, the idea of using polymeric cells to encapsulate transplanted cells (in particular islet cells) (FIG. 2), and to thereby protect them from immune rejection, was first proposed in the 1960s[1–3]. The method is based on a drop technique, in which a haemoglobin solution containing cells is added drop-wise to a silicone liquid containing diacid. The hydrophobic diacid only crosslinks haemoglobin at the interface of the two solutions, to form a membrane by interfacial polymerization[1]; the encapsulated cells in the resulting artificial cells thereby remain intact[2,3,14].

Although the idea of encapsulation of islets for the treatment of diabetes was proposed in the 1960s[1–3,14], it was not until 1980 that this idea was successfully



implemented[36]. In further experiments, Lim and Sun used a modification of the original drop technique — instead of chemically crosslinking protein to form the membrane, they used a milder electrostatic crosslinking of alginate and polylysine[36]; this alginate–polylysine–alginate method is now the most commonly used technique. When implanted into diabetic rats, micro-encapsulated islets corrected the diabetic state for several weeks[36].

Since 1980, many groups have been investigating this approach, or extensions of it, for islets. The results in animals seem promising[36–43], and the implanted encapsulated islets function for up to a year[36–43]. A study of a patient with type 1 diabetes by Soon-Shiong's group has shown that the procedure is safe and without side effects[4]. However, efficacy studies using larger quantities of encapsulated islets have not yet been carried out, as there are a number of challenges that need to be addressed before the initial clinical trials can be explored more extensively[38–43]. These challenges are so important that they will be discussed in detail in a later section.

Encapsulation of hepatocytes. Encapsulated hepatocytes have been used as a model for cell and gene therapy[44–52]. The implantation of encapsulated hepatocytes increases the survival of rats with fulminant hepatic failure[45]. Encapsulated rat hepatocytes are not rejected after implantation into mice; instead, there is an increase in viability after intraperitoneal implantation[46], owing to the retention of hepato-stimulating factors inside the microcapsules as they are secreted by the hepatocytes[47].

The Gunn rat is the model for the Crigler–Najjar syndrome in humans, which results from defects in the liver enzyme UDP-glucuronosyltransferase (UDPGT). Intraperitoneal implantation of artificial cells containing hepatocytes lowered the high systemic bilirubin levels that are characteristic of this condition[48,49]. Kinetic analysis shows that this is because of the presence of UDPGT in hepatocytes in artificial cells, which conjugated bilirubin to the monoconjugated and diconjugated form for excretion in urine, as in normal animals[50].

The most promising use of encapsulated hepatocytes is for short-term 'bridging' in acute liver failure to provide liver support while the patient's own liver regenerates and regains the capacity to function normally. However, this will probably have to be used in combination with haemoperfusion to first remove the large quantities of toxins and products released by the breakdown of the acutely damaged liver. Haemoperfusion has already been successfully used for removing these materials in fulminant hepatic failure, and resulted in the recovery of consciousness of patients in hepatic coma[31,32]. It is only after the removal of toxins that the implanted micro-encapsulated hepatocytes can carry out their function to maintain the patient alive, in the hope that the liver will regenerate sufficiently to take over[51,52].

Other areas. Other research has focused on the micro-encapsulation of erythropoietin (EPO)-secreting renal cells to treat anaemia[53], and parathyroid cells for secreting parathyroid hormone to treat hypoparathyroidism[54]. However, standard hormone-replacement therapy is more cost-effective at present for these conditions.

Challenge of cell availability. A key challenge for the approaches described above is the availability of human cells (allogeneic cells) for use in encapsulation. The use of xenogeneic cells from nonhuman sources has been proposed, because the polymeric membrane can exclude leukocytes and antibodies, which results in protection from the immune response [14,37,55]. For allografts that use human cells, immune mechanisms are mainly mediated by the interaction of lymphocyte sub-populations (specifically $CD8^+$ cells) with donor cells. So, the physical isolation provided by the capsules should prevent cell-to-cell contact between the encapsulated tissue and the host's immune system. However, research indicates that both xenografts and allografts might provoke an inflammatory cell response[56]. These results led to the proposal that cells in capsules produce proteins that can provoke inflammatory responses in immune cells, and that this even occurs with isografts. Another possibility is that the inflammatory cell response results from the use of the standard method for cell encapsulation, which leads to a few cells protruding on the surface of the artificial cells[57]; the use of the two-step method to prevent cell extrusion seems to prevent this problem[58–61] (FIG. 3). Both of these possibilities are important, and should be investigated further.

For xenografts using non-human cells, there are also other mediators that might provoke an immune response, including antibodies, complement fractions and cytokines[56,62]. Such an immune response could be problematic in the long term, and if this should prove to be the case, then it would mean that only human cells

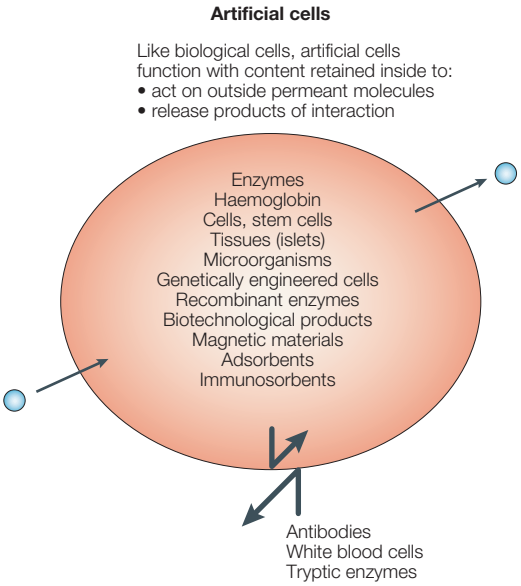


Figure 1 | **Polymeric artificial cells.** Many types of biologically active material can be micro-encapsulated in artificial cells individually or in combination[1–3,14,16,69]. Biologically active materials inside the artificial cells are prevented from coming into direct contact with external materials such as leukocytes, antibodies or tryptic enzymes. However, smaller molecules, such as hormones, peptides and small proteins, can equilibrate rapidly across the artificial cells, because of the large surface-to-volume ratio. By using different types of membrane material, the membrane porosity and therefore the permeability can be controlled over a wide range, from nonporous to highly porous and hydrophilic to lipophilic. Artificial cells can also range in size from macro-dimensions through micron- and nano-dimensions down to molecular dimensions (BOX 1). These possible variations allow for extensive variations in the properties and functions of artificial cells[1–3,14,16,69].

can be used. The use of xenogeneic cells might require the use of a membrane with very limited permeability[63–65]. Our group is using chromatography to study variations in the membrane composition of microcapsules and the effects they have on the diffusion of larger molelcules[66]. Other groups[67,68] showed that xenogeneic neonatal porcine islets, which are far less immunogenic than their adult counterparts, could be used to lessen the problems related to xenografts. Moreover, support mechanisms comprising either cells, or pharmacological agents co-encapsulated with xeno-cells, might help to attenuate the xeno-immune problem. However, there are also other sources of cells, as described below.

Genetically engineered cells. Cells can be genetically engineered outside the body to acquire very specialized and specific functions. However, the use of cells from the same person (autologous cells) is too individualized and therefore not suitable for scale-up production. The use of cells from other humans (allogeneic cells) is more suitable for scale-up production, but this requires the concomitant use of drugs to suppress immunological rejection of the foreign cells. As a result, there has been much research on the use of artificial cells that contain genetically engineered cells (TABLE 1)[69–71]. In this approach, the immunogenic foreign cells remain immuno-isolated within the artificial cell; however, oxygen and nutrients can still enter the genetically engineered cells, and therapeutic agents can diffuse out of the artificial cell and perform their function in the body (FIG. 2).

Encapsulated mouse fibroblasts carrying a human growth hormone (hGH) fusion gene continue to secrete and deliver hGH when implanted into an animal model of dwarfism[72]. Implantation of micro-encapsulated SK2 hybridoma cells that secreted anti-human interleukin-6 (IL-6) monoclonal antibodies (SK2 mAb) into transgenic mice (hIL-6 Tgm) suppressed immunoglobulin G1 (IgG1) plasmacytosis and significantly increased survival time in these mice[73]. Encapsulated genetically engineered mouse myoblasts that secrete human factor XI have also been studied for potential use in haemophilia B[70], and encapsulated myoblasts have been used to deliver EPO in a mouse model of β-thalassaemia[74].

Neurological disorders represent a potentially exciting area for the therapeutic application of polymeric artificial cells. For example, encapsulated neuro2A cells that contain the gene encoding proopiomelanocortin released endorphin and decreased sensitivity to pain when injected into the cerebrospinal fluid of rats[75,76]. In another example, encapsulated cells that secreted human nerve growth factor promoted the survival of AXOTOMIZED septal cholinergic neurons[77]. An extension of the principle of polymeric artificial cells is to prepare polymeric hollow fibres instead of spherical polymeric membrane artificial cells. An example of this approach is provided by the use of encapsulated baby hamster kidney (BHK) cells that contain the gene encoding human or mouse ciliary neurotrophic factor (CNTF) for treating the neurodegenerative disease amyotrophic lateral sclerosis[78]. Other examples[18,79,80] are highlighted in TABLE 1.

At present, regulatory agencies are not willing to approve the use of genetically engineered cells in humans unless no other treatment is available. In the case of Huntington's disease, a Phase I clinical trial was carried out using a semi-permeable membrane encapsulating a BHK cell line engineered to synthesize and release CNTF[79]. This was implanted into the right lateral ventricle of six subjects with stage 1 or 2 Huntington's disease; this membrane was retrieved and replaced every 6 months over a 2-year period. Improvements in electrophysiological measures of brain function were observed, and these were positively correlated with the amount of CNTF released. The authors reported that this Phase I study demonstrates the safety, feasibility and tolerability of this gene-therapy procedure. They also stressed the need to improve on the technique, as although all retrieved capsules were intact, they contained variable numbers of surviving cells, and CNTF release was low in 13 of 24 implants.

There has also been much recent interest in the potential use of polymeric artificial cells containing genetically modified cells for the treatment of tumours[19,20,81–83]. For example, encapsulated genetically modified cells expressing PRO-DRUG-activating enzymes, such as cytochrome P450, have been studied as a potential treatment for

AXOTOMIZE
Interruption of the axon of a neuron.

PRO-DRUG
A pharmacologically inactive compound that is converted to the active form of the drug by endogenous enzymes or metabolism.

ANGIOGENESIS
Growth of new blood vessels. For example, in pathology, the generation of a blood supply to a tumour.

inoperable pancreatic carcinoma in patients[83]. The encapsulated cells are administered intra-arterially, and accumulate in the pancreatic carcinoma, where they activate the drug ifosfamide. As a result, the local concentration of the activated ifosfamide is much higher than in the rest of the body, which reduces systemic toxicity and increases local efficacy. Encapsulated transfected cells that secrete the anti-ANGIOGENIC factor endostatin have also shown promise in animal models of malignant brain tumours[19,20]. Another approach is based on polymeric artificial cells that contain genetically modified myoblast cells that secrete IL-2 linked to the Fv region of a humanized antibody with affinity to tumours overexpressing the oncogene ERBB2[81]. As mentioned above, regulatory agencies are more likely to approve clinical trials for the use of genetically engineered cells for conditions that have no other effective treatments. Malignant tumours that have no available effective therapy are one typical example — as discussed above, clinical trials in inoperable pancreatic carcinoma in patients have been carried out[83]. Another example is the use of encapsulated transfected cells that secrete endostatin for malignant brain tumours[19].

The research described above has shown that polymeric artificial cells containing genetically modified cells have potential as therapeutics. However, further research is needed to study the long-term safety and effects of such systems to address regulatory concerns regarding the use of any products containing genetically modified cells. Until then, regulators are likely to be reluctant to allow clinical trials of genetically engineered cells in humans unless there is no other treatment available.

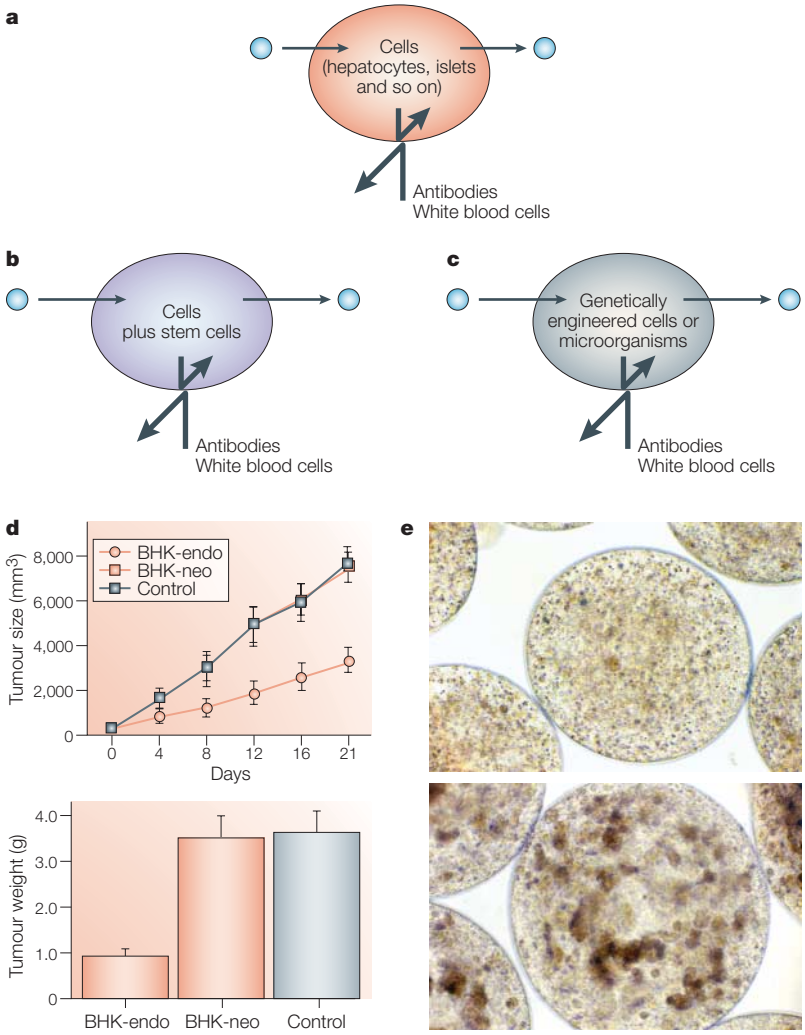


Figure 2 | **Potential therapeutic applications for polymeric artificial cells that encapsulate biological cells. a** | Islets retained inside artificial cells can secrete insulin into the body. The concentration of glucose in the body fluid is in equilibrium with the artificial cell content and can act as a feedback to adjust the amount of insulin that needs to be secreted[2–4,14,36–43]. Furthermore, the artificial cell membrane separates the islet from the external environment, thereby preventing immunological reactions. There are many other possible therapeutic applications afforded by the capacity of artificial cells to work in a similar manner with other cell types, such as hepatocytes[45–50]. **b** | Another promising area is the use of polymeric artificial cells containing cells and stem cells[59–61], because the stem cells can increase the viability and function of the cells after implantation. **c** | Polymeric artificial cells containing genetically engineered cells have also been studied for use in various conditions, including neurological diseases[71,77–80], haemophilia[70], cancer[19,20,81–83] and other conditions[72–76]. **d** | Shows the effects of encapsulated transfected cells that secrete endostatin (BHK-endo) for malignant brain tumours and the effects on tumour size[20]. **e** | The effects of 28 days in culture: encapsulated hepatocytes (top) are detached with decreased viability, whereas when co-encapsulated with whole bone-marrow stem cells (bottom), the hepatocytes remained attached and remained viable[59].

Stem cells. Even more recently, the use of stem cells[84,85] has been recognized as another possible therapeutic approach, because they can differentiate into different types of cell. Allogeneic stem cells still require immunoprotection inside polymeric artificial cells, but would not be beset by problems that affect xenografts. However, the quantity of stem cells that can be harvested for large-scale use is still limited at present. In order to increase the duration of viability and function of encapsulated hepatocytes after implantation, we have co-encapsulated hepatocytes with bone-marrow stem cells[59–61] (FIG. 2). This resulted in increased viability of the hepatocytes *in vitro* and *in vivo*, and also significantly prolonged the lowering of high systemic bilirubin levels in congenital Gunn rats with defects in the liver enzyme UDPGT[61]. Determining the underlying reason for this effect requires further investigation. However, it is known that bone-marrow stem cells in the presence of hepatocytes can differentiate into hepatocytes[84]. Furthermore, it has been proposed that bone-marrow stem cells secrete a factor that can help to maintain the viability of the co-encapsulated hepatocytes[59].

Encapsulating microorganisms. Another area is the study of artificial cells containing nonpathogenic microorganisms, including genetically engineered microorganisms. We have studied the encapsulation of a microorganism that removes cholesterol[86] and another that converts substrates to L-DOPA[87], and have also carried out research with fermentation induction of *Escherichia coli* with a *Klebsiella aerogenes* gene that increases the capacity for urea removal[88]. Recently, we have used metabolic induction to increase the urea-removal capabilities of *Lactobacillus delbrueckii* and have also encapsulated these microorganisms in artificial cells[89]. As microorganisms cannot be injected, we are studying the possibility of using oral microcapsules

Box 1 | **Dimensions of polymeric artificial cells**

Macro dimensions (<2 mm diameter)
- For genetically engineered cells, stem cells, other cells, tissues, microorganisms and so on.

Micron dimensions
- For enzymes, genetically engineered microorganisms and other microorganisms, peptides and so on.

Nano dimensions
- For blood substitutes, enzymes, peptides, magnetic materials, drugs and so on.

Molecular dimensions
- For blood substitutes, crosslinked enzymes, conjugated proteins and so on.

containing nonpathogenic microorganisms[88]. Our study shows that oral administration of polymeric artificial cells containing *Escherichia coli* with a *Klebsiella aerogenes* gene can lower elevated systemic urea levels in uraemic rats without any adverse effects[88]. It is important to be sure that any nonpathogenic microorganisms used this way would have no adverse effects after oral administration. Until then, it seems likely that regulatory agencies will require that there should be absolutely no leakage of genetically engineered microorganisms from orally administered polymeric microcapsules, even though they are classified as nonpathogenic. We are also developing the metabolic induction of *Lactobacillus delbrueckii* in artificial cells for possible use in urea removal in humans[89]. *Lactobacillus delbrueckii* has been used commonly in regulatory-agency approved food products for many years.

Other challenges for cell encapsulation. Many groups are looking into optimizing the biocompatibility, mass transfer, stability and reproducibility of polymeric artificial cells in cell encapsulation[5,6,90,91]. Long-term biocompatibility is very important, as foreign-body reaction and the resulting fibrous reaction will decrease mass transfer of oxygen, nutrients and metabolites and result in the death of the encapsulated cells. In addition, much work is being done to investigate structural–functional relationships in an attempt to increase the long-term stability of the polymeric artificial cells, and to achieve reproducible results[38–41,64]. There are a number of commercial machines for the automatic production of artificial cells, which have enhanced reproducibility and reduced variation in the size of the cells produced; artificial cells prepared using one such machine are shown in FIG. 3.

However, improving the reproducibility, biocompatibility and strength of the polymeric membrane might not be enough. When using the standard method to encapsulate high concentrations of cells, and even with automatic production, some cells are exposed on the surface or entrapped in the membrane matrix[57]. If cells are exposed on the surface, they are not immuno-isolated, and the rejection process would involve not only the exposed cells, but the whole artificial cell would be covered by the resulting infiltration of macrophages and fibroblasts. Entrapment of cells in the membranes weakens the membrane, which can eventually result in its breakage after implantation, thereby releasing the contents of the artificial cells (FIG. 3). So, for the encapsulation of high concentrations of small cells, we have designed a two-step method[58] (FIG. 3), which should be particularly useful for the encapsulation of hepatocytes and genetically engineered cells. This is based on the formation of very small alginate gel microsphere capsules containing cells. These small microspheres are enclosed within larger gel spheres before forming polymeric artificial cells, and then the small alginate gel microspheres are dissolved to release the cells. This way, the cells are dispersed freely inside the artificial cells, which obviates the requirement of an extra diffusion barrier (FIG. 3). Detailed studies comparing this approach to the standard methods show that this prevents the protrusion of cells from the membrane and therefore prevents local invasion of inflammatory cells, and also prolongs the viability of the implanted cells[58,60,61].

Other groups have attempted to use our earlier technology of enclosing smaller microcapsules inside larger microcapsules[2,3] or to use a double coating for the membrane. However, these approaches add further diffusion barriers. Another group has developed a promising approach that involves coating islets directly with a polymer membrane[5]. Many other extensions and modifications of the original method have also been studied[5], including the use of different types of membrane material[5]. Further extensions include the use of electrostatic devices[92], emulsification/thermal gelation[93] and JetCutter technology[94]. The use of high-voltage electrostatic pulses can produce much smaller alginate microcapsules (less than 300 μm), which would allow for better mass transfer than larger microcapsules[92]. The emulsification/thermal gelation method[93] can be used to prepare large batches of microcapsules more easily than present cell-encapsulation methods that involve variations of the drop approach. JetCutter technology[94] is another approach to solve the problems of the standard drop technique and can produce larger batches with greater ease.

Implantation of micro-encapsulated cells for extended periods is a long-term aim, but several groups are looking into other configurations for more immediate clinical applications. For example, Aebischer's ingenious use of capillary fibres to encapsulate cells has allowed his group to insert these subcutaneously into the cerebrospinal fluid on a short-term basis, and to remove and renew the fibres[95]. Such an approach would solve problems related to the permanent retention of genetically engineered cells and other effects of long-term implantation. However, the fibre-encapsulation and insertion approach is not applicable to conditions that require a large amount of encapsulated cells, as is the case for islets and hepatocytes. Another approach that does not require implantation or insertion is the oral administration of micro-encapsulated genetically engineered cells[88]. However, this is only applicable in certain conditions

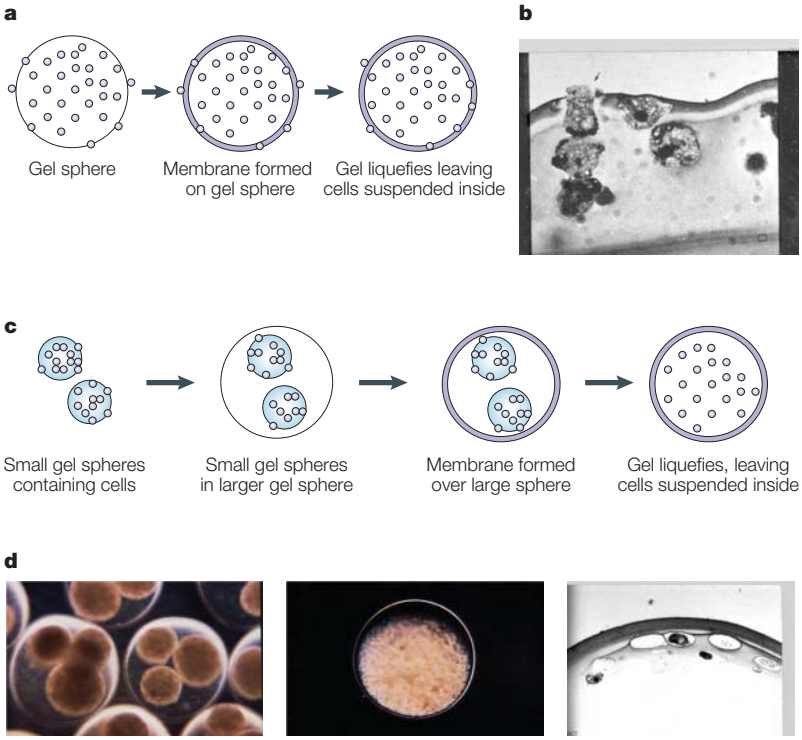


Figure 3 | **Development of artificial cells for cell encapsulation.** Interdisciplinary research is needed to develop cell encapsulation to a point at which it can be tested in large-scale clinical trials. **a**,**b** | Even with the most biocompatible membrane and automatic machines for producing artificial cells, some cells are still exposed to the surface, resulting in membrane instability and immune rejection[57,60]. **c**,**d** | One way to solve the problem of immune rejection is to use a two-step method, which prevents the encapsulated cells from being entrapped in the encapsulating membrane, which results in improved viability and function after implantation[58,60,61]. This method is based on the formation of very small alginate gel microsphere capsules containing cells. These small microspheres are then enclosed within larger gel spheres before forming polymeric artificial cells; the small alginate gel microspheres are then dissolved to release the cells. This way, the cells are dispersed freely inside the artificial cells, therefore avoiding an extra diffusion barrier. This prevents the protrusion of cells, prevents local invasion of inflammatory cells and also prolongs the viability of the implanted cells.

— for example, in the removal of unwanted metabolites from the body (phenylalanine in phenylketonuria (PKU), hypoxanthine in Lesch–Nyhan disease, tyrosine in melanoma and uraemic waste metabolites in kidney failure). For oral administration, very strong polymeric membranes that will not break down in the presence of the powerful digestive enzymes encountered in the intestine are needed. Furthermore, a buffer should be added to oral formulations to protect the enclosed cells on their passage through the acidic environment of the stomach. As with all medical therapies, different conditions will require different approaches for the use of encapsulated cells — either implantation, insertion or oral administration as appropriate.

Animal models make a key contribution in the assessment of efficacy of different types of therapy with encapsulated cells. The availability of many types of knockout models[96] will facilitate this greatly, although caution has to be exercised in extrapolating from results obtained with animals to humans[97].

Artificial cells in the micron dimensions

Enzyme therapy. In 1968, we implanted artificial cells containing catalase into animals with a congenital deficiency in catalase[12]. This replaced the deficient enzyme and prevented the animals from the damaging effects of oxidants; furthermore, the artificial cells protected the enclosed enzyme from immunological reactions[98]. It was also shown 3 years later that artificial cells containing asparaginase implanted into mice with lymphosarcoma delayed tumour growth[13]. However, unlike encapsulated cells that can continue to function, enzyme activity deteriorates at body temperature and repeated injections at weekly or more frequent intervals are therefore required. Furthermore, in the late 1960s and early 1970s, enzymes for the most common inborn error of metabolism, PKU, were only available as very complex and unstable liver enzymes that were not suitable for use in artificial cells for enzyme therapy. In addition, although of scientific interest, there has been no industrial incentive for developing this new therapeutic approach until recently. As discussed in the next section, with the availability of simple and stable enzymes derived from microorganisms, and with the demonstration of possible oral administration, enzyme therapy is now much more feasible and practical.

Oral administration and entero-recirculation. To solve the problem of the requirement for repeated injections, artificial cells were given orally. As they travel through the intestine, they act as microscopic dialysers. By encapsulating enzymes and other material inside the microcapsules, they can act as combined dialyser–bioreactors. For example, artificial cells containing urease and ammonia adsorbent were used to lower systemic urea levels[11]. Oral micro-encapsulated xanthine oxidase has been used to lower systemic hypoxanthine levels in a patient with Lesch–Nyhan disease, an extremely rare inborn metabolic disorder[99,100] (FIG. 4). Hypoxanthine is highly lipid soluble, and therefore equilibrates rapidly between the body fluid and the intestine. As Lesch–Nyhan disease is extremely rare, there is no commercial interest in developing the approach for routine clinical use. However, this preliminary demonstration of safety and effectiveness in a patient has encouraged the development of this approach for PKU.

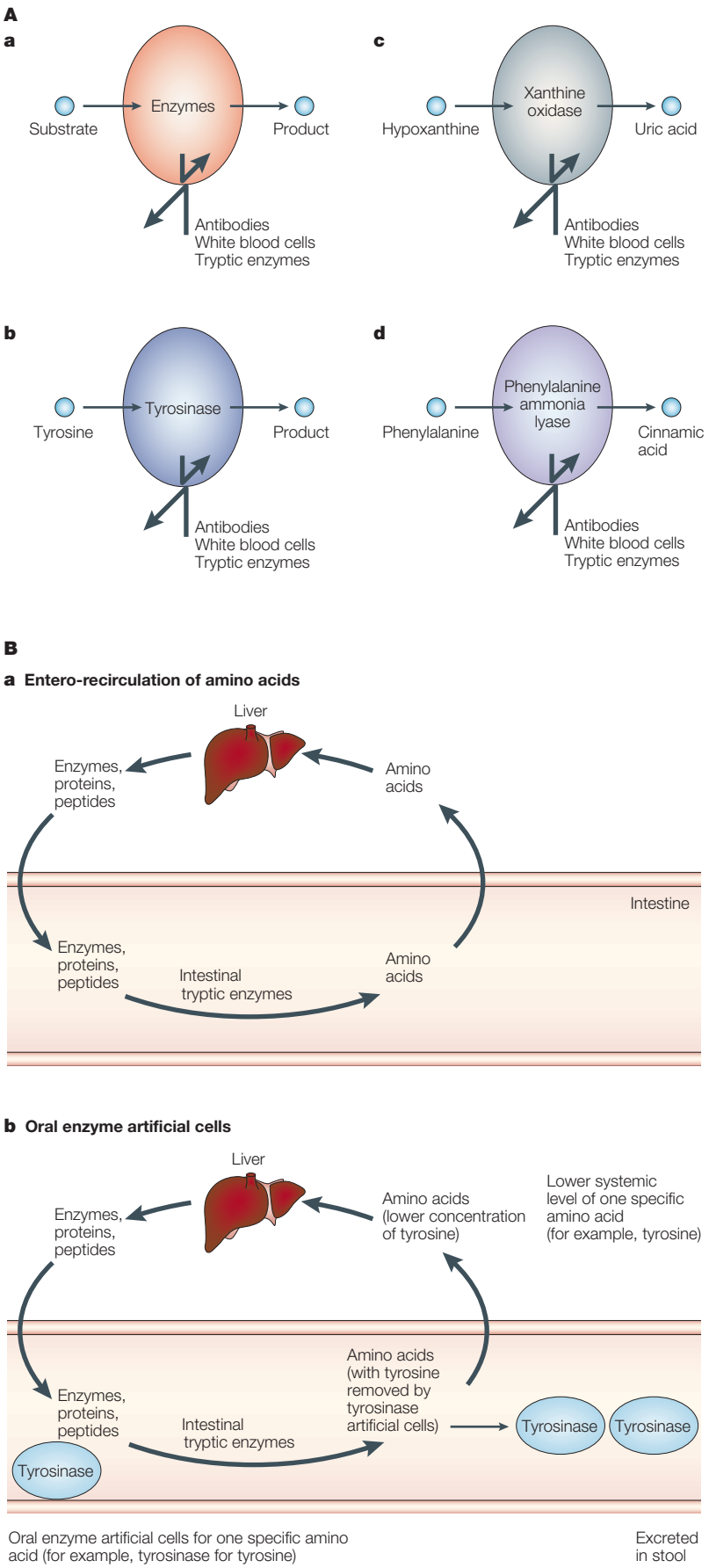
PKU is caused by a defect in a complex enzyme system in the liver that results in elevation of the systemic concentration of the amino acid phenylalanine to toxic levels. However, levels of amino acids in the intestine are many times higher than those in the body fluid. The standard theory is that protein in food is digested into amino acids, which results in a very high concentration of amino acids in the intestine. We found that the levels of amino acids in the intestine of rats on a 1-week protein-free diet are not significantly different from those on a standard diet containing protein[101]. This led to the proposal that the source of the high level of amino acids in the intestine is as follows[101]. It is well known that large amounts of gastric, pancreatic and intestinal juice are secreted daily into the gastrointestinal tract. These

secretions contain high concentrations of enzymes and other proteins and peptides, which are in turn digested in the intestine into amino acids. These are reabsorbed into the body, and then synthesized by the liver into proteins and peptides, some of which are secreted again into the intestine. This would result in an extensive entero-recirculation of amino acids between the body and the intestine[101] (FIG. 4). If this is the case, then the oral administration of artificial cells containing one specific enzyme (for example, phenylalanine ammonia lyase) for one specific amino acid (for example, phenylalanine) would cause the removal of the amino acid from the entero-recirculation, resulting in a decrease in the systemic concentration of this particular amino acid (FIG. 4). This would explain the finding that artificial cells containing phenylalanine ammonia lyase given orally can lower the elevated phenylalanine levels in PKU rats to normal levels[102]. Furthermore, unlike the control group, the animals continued to gain weight and grow. A key challenge with this application is the

Table 1 | **Polymeric artificial cells containing cells**

Cell content	Aim	Comment on clinical development	References
Cells and tissues			
Pancreatic islets	Feedback controlled secretion of insulin for diabetes mellitus	Tested in Phase I trial. Further development of encapsulation technology needed before larger trials can be conducted	2–4,14, 36–43
Hepatocytes	To support liver function in liver failure	Being developed for use in combination with haemoperfusion for fulminant hepatic failure	45–50
Kidney cells	To secrete erythropoeitin to treat anaemia	Standard replacement therapy more cost-effective at present	53
Parathyroid cells	To secrete parathyroid hormone to treat hypoparathyroidism	Standard replacement therapy more cost-effective at present	54
Hybridomas	Antibody production		5,6,34
Genetically engineered cells			
Mouse myoblast (*mGH* gene) SK2 Hybridoma cells	Secrete mGH for dwarfism Secrete anti-hIL-6 monoclonal antibodies for IgG1 plasmacytosis	Regulatory agencies are less likely to approve the use of genetically engineered cells for human use unless there are no alternatives available	72 73
Mouse myoblasts (human factor IX gene)	Secretes human factor IX for haemophilia B		70
Hamster kidney cells (*CNTF* gene)	Secrete ciliary neurotrophic factor for amyotrophic lateral sclerosis		78
Neuro2A cells (pro-piomelanocortin gene)	Secrete β-endorphin for pain treatment		75,76
Hamster kidney fibro-blasts (*hNGF* gene)	Secrete human nerve growth factor (hNGF) for Parkinsonism		70
Xenogeneic BHK cells (*hNGF* gene)	Secrete human nerve growth factor (hNGF) for axotomized septal cholinergic neurons		77
Cells producing CNTF	To alleviate Huntington's disease	Phase I clinical trial	78, 79
iNOS-expressing cells	Tumour suppression	Clinical trials in patients with inoperable pancreatic cancinoma	83
IL-2-secreting C2C12 cells	Tumour suppression	Effective in animal study	81
Stem cells			
Stem cells plus hepatocytes	Increase duration of viability and function of hepatocytes	Being developed for shorter-term use in combination with haemoperfusion for fulminant hepatic failure to allow the liver time to regenerate	59–61
Microorganisms			
Pseudomonas pictorum	To remove cholesterol	*In vitro* studies	86
Erwinia herbicola	To convert ammonia pyruvate and phenol into L-lysine and L-DOPA	*In vitro* studies	87
CDH5 *Escherichia coli*	Urea removal	Effective in uraemic rats but used genetically engineered cells	88
Metabolic induction of *Lactobacillus delbrueckii*	Urea removal	*In vitro* studies; still to be developed for clinical use	89

BHK, baby hamster kidney; CNTF, ciliary neurotrophic factor; hNGF, human nerve growth factor; IL, interleukin; iNOS, inducible nitric oxide synthase.



high cost of phenylalanine ammonia lyase; however, two groups are now developing recombinant phenylalanine ammonia lyase that should be less costly[104].

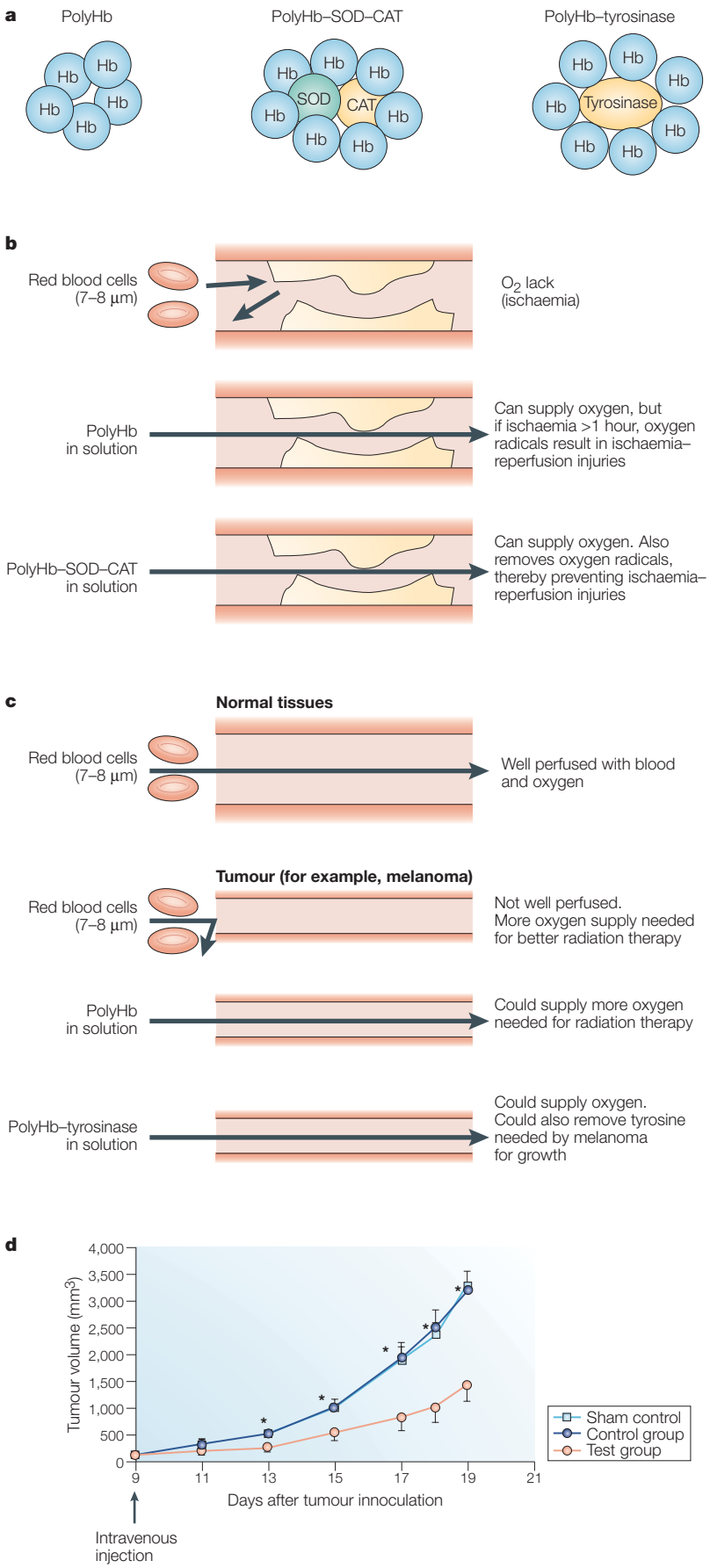
If the proposal of extensive entero-recirculation of amino acids is correct[101], then any specific amino acid in the body can be removed by the oral delivery of the specific enzyme for the specific amino acid (FIG. 4). We tested this hypothesis by orally administering polymeric artificial cells containing tyrosinase to rats[105], and showed that systemic tyrosine can be lowered by daily oral administration in this way[105]. Part of the motivation for this study was that it has been shown that severe dietary restriction of tyrosine can delay the spread of the skin cancer melanoma in an animal model[106]. However, such dietary restriction also results in severe weight loss and other adverse effects, and so could not be tested in humans. Results with oral administration of tyrosinase artificial cells show that rats continue to gain weight at the same rate as the control group and that there is no adverse effect[105].

Biodegradable polymeric artificial cells. In order to solve the problem of the accumulation of enzyme artificial cells from repeated injections, we prepared biodegradable polylactic acid artificial cells[9]. The polylactic acid polymer can degrade in the body into lactic acid, and finally into water and carbon dioxide. These cells can be made to contain hormones, enzymes and other proteins[9]. This approach is now used more for the delivery of drugs[21–23,107] and hormones[108,109].

Artificial cells with molecular dimensions

Red-blood-cell substitutes based on polyhaemoglobins. The development of blood substitutes is a large area that has been reviewed recently elsewhere[110–113]. The original idea of artificial red blood cells of micron dimensions[1–3,11,14] has been extended to a simpler polyhaemoglobin — a molecular version of artificial red

Figure 4 | **Applications of artificial cells containing enzymes. Aa** | Artificial cells containing enzymes can work to remove or convert specific systemic substrates crossing into the artificial cells. The enzymes are prevented from coming into direct contact with external antibodies, tryptic enzymes or white blood cells[1–3,12–14]. **b** | Polymeric artificial cells containing tyrosinase given orally can lower systemic tyrosine needed for the growth of the skin cancer melanoma[105]. **c** | Polymeric artificial cells containing xanthine oxidase have been given orally to a patient with Lesch–Nyhan disease to lower systemic levels of hypoxanthine[99,100]. **d** | Polymeric artificial cells containing phenylalanine ammonia lyase given orally lowers elevated phenylalanine levels in rats with phenylketonuria[102]. **Ba** | Proposal for entero-recirculation of amino acids. Pancreatic, gastric and intestinal secretions contain high concentrations of enzymes and other proteins and peptides. These are digested by intestinal tryptic enzymes into amino acids that are reabsorbed into the body, after which the liver uses them for protein synthesis. These are then secreted as above into the intestine and the cycle is repeated continuously[101]. **b** | By giving polymeric artificial cells containing one specific enzyme (for example, tyrosinase), the recycling of a specific amino acid (for example, tyrosine) can be interrupted. This can be used to lower the systemic level of one specific amino acid[101,102,105].



blood cells (FIG. 5). This is based on the use of bifunctional agents such as diacid[1,2,11], or later glutaraldehyde[17], to crosslink haemoglobin molecules into soluble polyhaemoglobin (FIG. 5). However, there was little interest in this idea until recent problems of donor blood that was contaminated with HIV and hepatitis C emerged, which has resulted in a drive to develop blood substitutes.

The urgent need for blood substitutes meant that clinical development of all types of blood substitute was pursued, but the speed with which they were required resulted in the omission of some of the required basic research into their usefulness. As a result, many approaches subsequently failed in humans. Two of the most successful approaches are based on glutaraldehyde-crosslinked polyhaemoglobin. These have been developed independently by two groups, based on Chang's basic principle of glutaraldehyde-crosslinked polyhaemoglobin[17]. One approach uses pyridoxalated glutaraldehyde human polyhaemoglobin[114,115], which in Phase III clinical trials successfully compensated for extensive blood loss in trauma surgery by maintaining the haemoglobin level, with no reported side effects[114]. Individual trauma surgery patients have received up to 10 litres of polyhaemoglobin, and a clinical trial involving 141 patients using polyhaemoglobin in trauma surgery has been completed and published[114]; FDA approval is now awaited.

The second polyhaemoglobin is glutaraldehyde-crosslinked bovine polyhaemoglobin, which has been extensively tested in Phase III clinical trials in perioperative surgery[116,117]. This bovine polyhaemoglobin has been approved for veterinary medicine in the

Figure 5 | **Artificial cells in the molecular dimensions.** **a** | Artifical cells as oxygen carriers. Glutaraldehyde can polymerize haemoglobin (Hb) into soluble polyhaemoglobin (polyHb) that continues to carry and deliver oxygen[17,114,116]. This strategy is in the final stages of Phase III clinical trials to replace blood loss in surgery in the United States and is in routine use for patients in South Africa and Russia[114–117]. **b** | In conditions involving a sustained lack of oxygen (for example, haemorrhagic shock, stroke and organ transplantation) the use of polyHb alone can cause ischaemia–reperfusion injury owing to the formation of oxygen radicals. To circumvent this, polyHb is crosslinked together with small amounts of superoxide dismutase (SOD) and catalase (CAT). PolyHb–SOD–CAT has been used to supply oxygen and at the same time remove oxygen radicals, thereby avoiding ischemia–reperfusion injuries[110,113,119–121]. **c** | Potential application of polyHb to aid cancer therapy. The blood supply to tumour tissue is limited, but chemotherapy and radiation therapy work better with higher oxygen tension[117,123]. PolyHb can perfuse tumour tissue better than red blood cells. Crosslinking polyHb with tyrosinase to form polyHb–tyrosinase has the added advantage of lowering systemic tyrosine needed for the growth of the skin cancer melanoma[124,125]. **d** | Effects of daily intravenous polyHb–tyrosinase in mice on growth of B16F10 melanoma in mice[125]. Light blue: sham control; dark blue: saline control (0.1 ml intravenous saline daily); orange: test group (0.1 ml intravenous polyHb–tyrosinase daily). *Statistically significant (P <0.05). Endpoint of study was when tumour in any one group reached 10% of normal body weight.

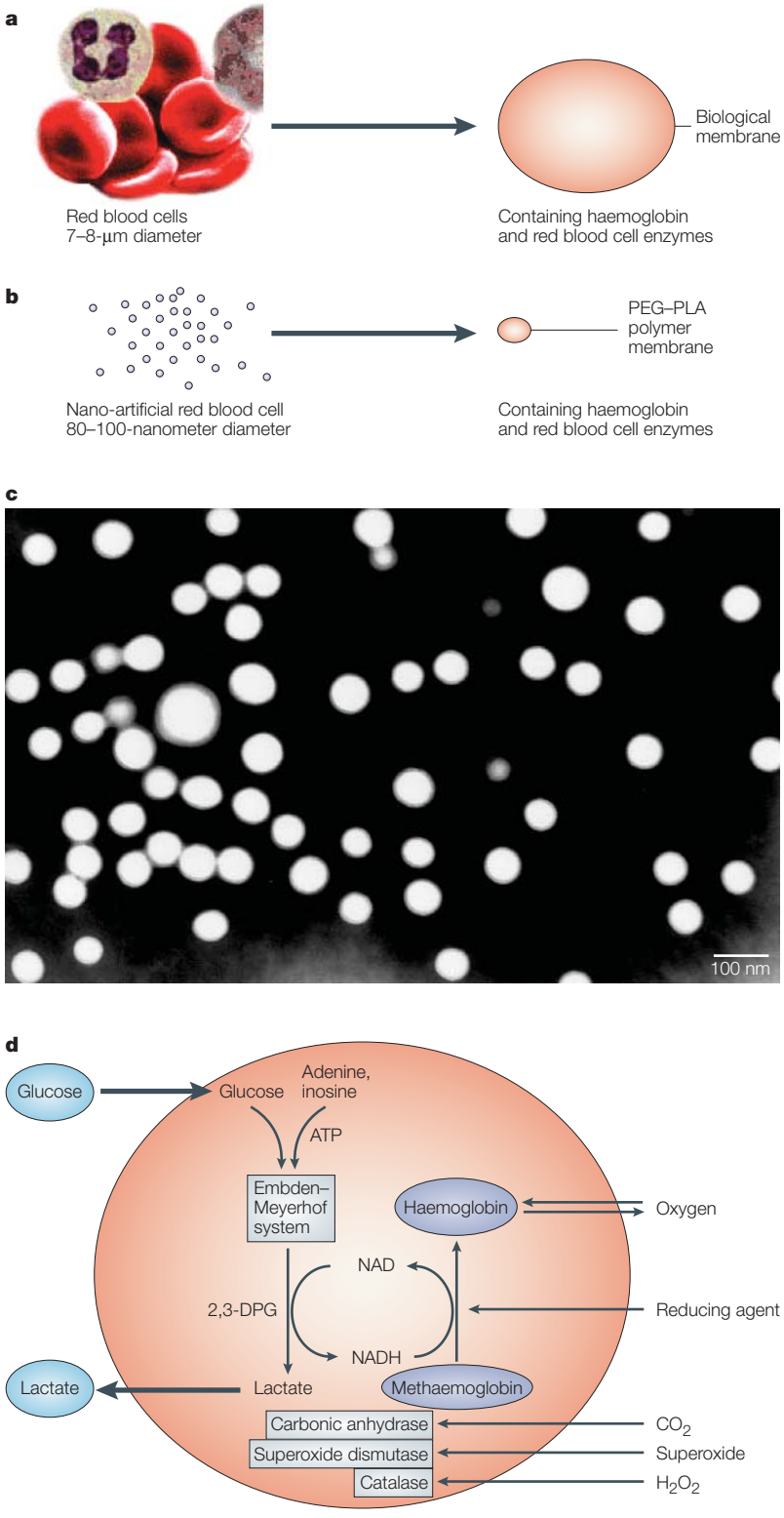


Figure 6 | **Artificial cells with nano-dimensions. a,b** | Red blood cells can circulate freely to carry out their functions. Polymeric artificial red blood cells can be prepared, but they have to be in the nano-dimension and with special membrane materials (for example, polyethylene glycol–polylactide (PEG–PLA) copolymer) in order for them to circulate freely[131,132]. **c** | Typical nano-dimension artificial red blood cells of 80-nm mean diameter. **d** | Except for the nano-dimension and the biodegradable polymeric membrane, these artificial red blood cells can be made to contain all the haemoglobin and enzymes of a red blood cell, with all its biochemical properties[132].

United States, and for routine clinical use in patients in South Africa. In the United States, the above two polyhaemoglobins have been approved for compassionate uses in humans and are awaiting regulatory approval for routine clinical use in humans. Another glutaraldehyde polyhaemoglobin is in routine use in patients in Russia.

Polyhaemoglobins have a number of advantages when compared with donor red blood cells. Unlike red blood cells, there is no issue of blood-group-compatibility for polyhaemoglobins, and they can therefore be given to patients immediately, and without time-consuming typing and cross-matching. They are also free from infectious agents such as HIV, hepatitis C, bacteria, parasites and so on. Furthermore, whereas donor blood has to be stored at 4°C, and can only be stored for 42 days, polyhaemoglobins can be stored at room temperature for more than a year. However, polyhaemoglobin is only an oxygen carrier, and does not have the same enzyme activity as red blood cells, which might be needed for certain clinical conditions. These include those for which ischaemia-reperfusion injuries are a potential concern, such as sustained haemorrhagic shock, stroke, myocardial infarction and organ transplantation. Furthermore, the circulation half-life of polyhaemoglobin is ~24 hours.

Polyhaemoglobins crosslinked with red-blood-cell antioxidant enzymes. Polyhaemoglobins can be kept at room temperature and used immediately in an emergency, and could therefore have considerable potential for treating severe haemorrhagic shock. However, if there is a substantial delay, and if the haemorrhagic shock is severe, reperfusion using an oxygen carrier alone might result in ischaemia–reperfusion injuries due to the production of oxygen radicals[110,113,118].

We are studying the use of crosslinked polyhaemoglobin–superoxide dismutase–catalase (PolyHb–SOD–CAT) [110,113,119–121] (FIG. 5). The intestine is one of the organs that is most likely to be injured in this type of ischaemia–reperfusion injury, and, unlike polyHb, polyHb–SOD–CAT did not cause a significant increase in oxygen radicals when it was used to reperfuse ischaemic rat intestine[110].

The obstruction of arteries due to thrombosis or other causes can result in a range of conditions, including stroke and myocardial infarction. Polyhaemoglobin is in solution and is therefore more likely to be able to perfuse partially obstructed vessels compared with red blood cells. However, if arterial obstruction and lack of oxygen is prolonged, reperfusion with an oxygen carrier alone could also result in ischaemia–reperfusion injuries. For example, after 60 minutes of ischaemia in a rat stroke model, reperfusion with polyHb resulted in a significant increase in the breakdown of the blood– brain barrier and an increase in brain oedema[113,122]. On the other hand, polyHb–SOD–CAT did not result in these adverse changes[113,122]. So, polyhaemoglobin or other oxygen carriers are well suited for certain conditions, especially for surgery. However, oxygen carriers with antioxidant properties should be used in other conditions for which there is potential for ischaemia– reperfusion injuries.

Polyhaemoglobin crosslinked with tyrosinase. Malignant tumours are well vascularized but have abnormal microcirculation, which results in under-perfusion by red blood cells and therefore lower tissue oxygen tension[117]. Attempts have been made to decrease the blood supply even further by the use of agents that inhibit angiogenesis to prevent the growth of blood vessels to the tumour; however, radiation therapy[123] and chemotherapy[117] work better with better tissue oxygen tension. As polyhaemoglobin is in solution, it can perfuse the abnormal microcirculation of tumours more effectively than red blood cells and therefore supply more oxygen. Furthermore, as polyhaemoglobin stays in the circulation for only 24 hours, it has a short duration of action and so only functions during radiation therapy. Polyhaemoglobin has been shown to decrease the rate of tumour growth and increase the lifespan in a rat model of gliosarcoma when used as an adjunct for chemotherapy[117]. We have recently crosslinked tyrosinase with haemoglobin to form a soluble polyhaemoglobin–tyrosinase complex[124,125] (FIG. 5). This has the dual function of supplying the oxygen needed for optimal chemotherapy or radiation therapy and also of lowering systemic levels of tyrosine. We have shown that intravenous injections of polyhaemoglobin–tyrosinase can delay the growth of the melanoma without causing adverse effects or changes in the growth of the treated animals[125]. Intravenous injections of polyhaemoglobin–tyrosinase can be combined with oral administrations of microencapsulated tyrosinase to maintain a low systemic tyrosine level[126].

Conjugation of polymer with proteins. Another way to form polymeric artificial cells on a molecular scale is by the crosslinking of protein to polymer[1–3,11]. The crosslinking of enzymes to a soluble polymer to form polymeric therapeutics is of particular interest, and has recently been reviewed in detail[23]. The crosslinking of haemoglobin to a soluble polymer is another approach towards the development of blood substitutes on a molecular level; this method is at an earlier stage of clinical trials, but has generated promising results[112].

Nano-dimension artificial cells

Polyhaemoglobin is in the final stages of Phase III clinical trials in the United States, in routine use in South Africa and Russia, and research is ongoing towards more complete red blood cell substitutes. Modifying the method of preparing micro-dimension polymeric artificial cells[1] can result in nano-dimension artificial cells. In the case of blood substitutes, nano-dimension liposomes of about 200 nm that contain haemoglobin have been prepared[127–129]. As this review deals with polymeric artificial cells, readers are referred to other sources for this topic[127–129]. Biodegradable polymer membranes are being used to form nano-dimension artificial red blood cells as a third-generation blood substitute[110,113,130–132]. These nano-dimension artificial RBCs (80–150 nm in diameter) contain all the red-blood-cell enzymes[110,113,132] (FIG. 6). Recent studies show that using a polyethylene-glycol–polylactide copolymer membrane, it is possible to increase the circulation time of these nano-dimension artificial red blood cells to double that of polyhaemoglobin[132]. Further studies in rats showed that one infusion with a volume of one-third the total blood volume did not result in any adverse effects or changes in histology or blood biochemistry when examined on days 1, 7 and 21 after infusion. Although not part of the present review, it should be mentioned that nano-dimension polymeric artificial cells are also being used in drug delivery for biologically active proteins, peptides and drugs[25,133].

Concluding remarks

Polymeric artificial cells containing bioadsorbent have been in routine clinical use for several years. The use of polymeric artificial cells for drug delivery is also relatively straightforward, and there are some routine clinical applications of such cells[21–23]. The oral administration of polymeric artificial cells containing enzymes is safe and effective, but less expensive enzymes are needed before their widespread clinical use becomes possible. First-generation blood substitutes in the form of polyhaemoglobin are already in routine use in some countries for surgery, but new generations of blood substitutes need to be developed for other conditions.

More complicated applications of artificial cells, such as cell encapsulation, have considerable therapeutic potential. As discussed in a recent consensus publication[6], significant progress has been made toward the successful transplantation of encapsulated cells in experimental animal models, which has resulted in promising potential therapies for a number of conditions. However, there are major challenges to be resolved before meaningful large-scale clinical trials can be carried out. Key requirements include reliable and safe sources of cells, biocompatible and stable membranes with suitable molecular cut-off to prevent immune rejection, reproducibility of the product and long-term survival of the graft. In addition, the use of xenografts or genetically engineered cells raises additional ethical, political and regulatory questions that need to be resolved. To promote the development of the routine clinical applications of polymeric artificial cells, we need to change our attitudes towards these entities. The impression that all polymeric artificial cells involve very simple technologies that can be easily developed for clinical use emerged, but we now know that this is not the case. In fact, we have to approach their use in a highly interdisciplinary manner, integrating basic and applied research and development that involves polymer chemistry, cell physiology, molecular biology, biotechnology and other areas[6,113]. This interdisciplinary approach will accelerate the clinical realization of the many exciting potential applications, bringing us towards a new era in which polymeric artificial cells are combined with advances in biotechnology and molecular biology to deliver new therapeutics.

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Acknowledgements

This author acknowledges the supports of the Canadian Institutes of Health Research, the 'Virage' Centre of Excellence in Biotechnology from the Quebec Ministry, the MSSS-FRSQ Research Group award on Blood Substitutes in Transfusion Medicine from the Quebec Ministry of Health's new Haemovigillance and Transfusion Medicine Programme and the Operating grant from the Research Fund of the Bayer/Canadian Blood Agency/Haema Quebec/Canadian Institutes of Health Research.

Competing interests statement

The author declares no competing financial interests..

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