ISBP AWARD LECTURE

ARTIFICIAL CELL BIOTECHNOLOGY FOR MEDICAL APPLICATIONS

(published in Blood Purif 18:91-96, 2000. Updated September 2002)

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Keywords

Oral removal uremic metabolites. Hemoperfusion. Enzyme therapy. Cell therapy. Gene therapy. Blood substitutes. Bioencapsulation. Artificial Cells

Abstract:

Artificial cells are prepared in the laboratory for medical and biotechnological applications. The earliest routine clinical use of artificial cells is in the form of coated activated charcoal for hemoperfusion. Implantations of encapsulated cells are being studied for the treatment of diabetes, liver failure and the use of encapsulated genetically engineered cells for gene therapy. We recently found that daily orally administered artificial cell containing a genetically engineered microorganism can lower the elevated urea level in uremic rats to normal levels and increase the survival of the animal. Furthermore, this can remove potassium, phosphate, uric acid and other waste metabolite from uremic plasma. Blood substitutes based on modified hemoglobin are already in Phase III clinical trials in patients with as much as 20 units infused into each patient during trauma surgery. Artificial cells containing enzymes are being developed for clinical trial in hereditary enzyme deficiency diseases and other diseases. Artificial cell is also being investigated for drug delivery and for use in other uses in biotechnology, chemical engineering and medicine.

Introduction

Artificial cell evolves from Chang's attempts to prepare artificial structures for possible replacement or supplement of deficient cell functions [1-5]. Like natural cells, biologically active materials inside the artificial cells are retained and prevented from coming into contact with external materials like leucocytes, antibodies or tryptic enzymes. Molecules smaller than protein can equilibrate rapidly across the ultrathin

membrane with large surface to volume relationship. A number of potential medical applications using artificial cells have been proposed [2-5]. The first of these developed successfully for routine clinical use is hemoperfusion [6]. After initial clinical trails in the form of coated activated charcoal hemoperfusion for poisoning, kidney failure, and liver failure [6], it is now in routine clinical uses [7]. This review will highlight some examples of the increasing interests in the biotechnological approaches of artificial cells for clinical applications. Some exciting recent developments include research and clinical trials on modified hemoglobin for blood substitutes; the use of artificial cells for enzyme therapy, cell therapy and gene therapy [8]. The use of oral artificial cells containing genetically engineered cells in uremia is a new area of potential interest to nephrologists.

Artificial cells containing enzymes for inborn errors of metabolism and other conditions

Chang and Poznanksy have earlier implanted artificial cells containing catalase into acatalesemic mice, animals with a congenital deficiency in catalase [9]. This replaces the deficient enzymes and prevented the animals from the damaging effects of oxidants. The artificial cells protect the enclosed enzyme from immunological reactions [10]. Chang also showed that artificial cells containing asparaginase implanted into mice with lymphosarcoma delayed the onset and growth of lymphosarcoma [11]. The single problem preventing the clinical application of enzyme artificial cells is the need to repeatedly inject these enzyme artificial cells. To solve this problem, Bourget and Chang found that microencapsulated phenylalanine ammonia lyase given orally can lower the elevated phenylalanine levels in phenylketonuria[PKU] rats [12]. This is because of our more recent finding of an extensive recycling of amino acids between the body and the intestine [13]. This is now being developed for clinical trial in PKU [14]. In addition to PKU other examples include the removal of tyrosine in tyrosinemia or melanoma, the removal of glutamine or asparagine in other cases. We are encouraged in this oral approach because of our preliminary clinical testing of oral microencapsulated xanthine oxidase as experimental therapy in Lesch-Nyhan Disease[15].

Artificial cells encapsulated cells for cell therapy

Chang et al reported the encapsulation of biological cells in 1966 based on a drop method and proposed that "protected from immunological process, encapsulated endocrine cells might survive and maintain an effective supply of hormone" [3,5]. Lim and Sun developed this drop-method by using milder physical crosslinking [16]. This resulted in alginate-polylysine-alginate [APA] microcapsules containing cells. They show that after implantation, the islets inside artificial cells remain viable and continued to secrete insulin to control the glucose levels of diabetic rats. Many groups are now developing cell encapsulation for cell therapy. This includes artificial cells containing endocrine tissues, hepatocytes and other cells for cell therapy [8,17-21]. Implantation of microencapsulated genetically engineered cells has been reviewed recently [21]. This has been studied for potential applications in amyotrophic lateral sclerosis, Dwarfism, pain treatment, IgG_1 plasmacytosis, Hemophilia B, Parkinsonism and axotomized septal cholinergic neurons.

The promising results of implantation of encapsulated cells require further developments especially to improve the long term biocompatibility for implantation. In the meantime, several groups are looking into other configurations and other sites of action for more immediately clinical applications. For example, Aebischer et al's ingenious use of capillary fiber to encapsulate cells has allowed his group to implant cells followed by retrieval and re-implantation in clinical trials [22]. In those conditions where larger amount of cells are needed, e.g. islets and hepatocytes, other approaches have been developed. Thus, Humes' group uses "encapsulated" renal tubule cells with capillary fibres for a "bioartificial kidney"[19]. Dimetrious et al [19] are testing hepatocytes "encapsulated" in a capillary fiber device [19] in series with a encapsulated charcoal hemoperfusion device for liver failure patients [19]. Because of long-term blood compatibility problems, both of these can only be used in acute conditions. Another approach is using oral administration as described below

Does orally administered artificial cells containing genetically engineered cells have a role in uremia therapy?

Advances in molecular biology have resulted in the availability of nonpathogenic genetically engineered microorganisms that can effectively use uremic metabolites for cell growth. Prakash and Chang therefore studied the oral use of microencapsulated genetically engineered nonpathogenic E.coli DH5 cells containing Klebsiella aerogenes urease gene in renal failure rats [23-25].

Red Blood Cell Substitutes

Polyhemoglobin as blood substitutes: Native hemoglobin [tetramer], breaks down into half molecules [dimers] after infusion causing renal toxicity and other adverse effects. Chang has extended his original approach of artificial cells containing hemoglobin and enzymes[1,2] to form polyhemoglobin – a molecular version of artificial cells. This is based on the use of bifunctional agents like diacid [2,4] or glutaraldehyde [35] to crosslink hemoglobin molecules into polyhemoglobin. This gluataradehyde crosslinked polyhemoglonin approach has been extensively developed more recently [36-41]. Polyhemoglobin consisting of 4 to 5 hemoglobin molecules stays longer in the circulation and they do not breakdown into dimers. One example is the recent report by Gould et al on their ongoing clinical trials using pyridoxalated glutaraldehyde human polyhemoglobin in trauma surgery. They show that this can successfully replace blood loss by maintaining the hemoglobin level with no reported side effects[37,38]. More recently, they have infused up to 20 units into individual trauma surgery patients. (2002 Sept update: Gould et al has published their Phase III clinical trial results for trauma surgery in the 2002 July issue of J. American College of Surgeons. Biopure's glutaraldehyde crosslinked bovine hemoglobin has been approved for human use in South Africa in April 2001) An o-raffinose polyhemoglobin has also been developed.

Polyhemoglobin containing catalase and superoxide disumutase: The present polyhemoglobin shows promise especially for perioperative uses as in hemodilution, replacement of extensive surgical blood loss and other conditions with no potentials for ischemia-reperfusion injuries[40]. However, polyhemoglobins do not contain red blood

cell antioxidant enzymes like catalase and superoxide dismutase. Thus, for the resuscitation of sustained severe hemorrhagic shock or in reperfusion of ischemic organs as in stroke or in organ trasnplantation, the use of polyhemoglobin may result in ischemia-reperfusion injuries [43]. D'Agnillo and Chang have therefore studied the crosslinking of superoxide dismutase and catalase with polyhemoglobin to form PolyHb-SOD-CAT [44]. We found that when compared to polyhemoglobin, PolyHb-SOD-CAT, significantly decrease the release of heme and iron from hemoglobin and also effectively removes oxygen radicals [44,45]. Reperfusion studies in a rat model of intestinal ischemia, shows that PolyHb-SOD-CAT resulted in negligible increase in oxygen radicals, unlike the high level that resulted from reperfusion using polyhemoglobin [46].

Recombinant human hemoglobin: Although polyhemoglobin is in the most advance stages of clinical trial, there are other modified hemoglobins [40,41, 47-49]. Unlike polyhemoglobin these are single tetrameric hemoglobin formed by intramolecular cross-linkage [50,51] or recombinant human hemoglobin [52,53]. Clinical trials on these show vasoactivities and other effects of nitrate oxide removal [51,53]. Lemon's group [54] has therefore developed a new recombinant human tetrameric hemoglobin with markedly decrease affinity for nitric oxide [54]. When infused into experimental animals, this did not cause vasoactivity.

Other new generations of modified hemoglobin blood substitutes: Polyhemoglobin stays in the circulation with a half-time of only up to 27 hours. In order to increase this circulation time, Chang's original idea of a complete artificial red blood cell [1,2] is now being developed as third generation blood substitute. Thus submicron lipid membrane microencapsulated hemoglobin [55] is being explored especially more recently by the group of Tsuchida in Japan [49] and Rudolph in the U.S.A.[48]. The U.S. group has modified the surface properties to result in a circulation half-time of about 50 hours [56]. Chang and Yu are developing a new system based on biodegradable polymer and nanotechnology resulting in polylactide membrane hemoglobin nanocapsules of about 150 nanometre diameter [57-61]. This is smaller than the lipid-vesicles and contains negligible amounts of lipids. We have included superoxide dismutase, catalase and also multienzyme systems to prevent the accumulation of methemoglobin. The circulation time is double that of polyhemoglobin

General:

The above review contains a very brief overview of this rather large area. For more specific details, please refer to the references given including those published recently from here (62-65). Artificial Cells Biotechnology is a rapidly evolving area and rapidly updating can be found at our McGill University website: www.artcell.mcgill.ca

Acknowledgements

The supports of the Medical Research Council of Canada, now Canadian Institute for Health Research, the "Virage" Centre of Excellence in Biotechnology from the Quebec Ministry and other sources of support are all gratefully acknowledged.

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