Serum-free Cell Culture: The Serum-free Media Interactive Online Database

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Summary

Fetal bovine serum (FBS) is a ubiquitously used essential supplement in cell culture media. However, there are serious scientific and ethical concerns about the use of FBS regarding its harvest and production. During the last three decades, FBS could be substituted by other supplements or by the use of defined chemical components in serum-free cell culture. A number of serum-free medium formulations have been described for mammalian and insect cell lines as well as for primary cultures. However, the switch to serum-free media still demands a time-consuming literature survey and a manufacturer search for appropriate medium formulations, respectively. Here we present the second collection of commercially available serum-free media in an updated, freely accessible interactive online database. Searches for serum-free media and continuous cell lines already adapted to serum-free culture can be performed according to various criteria. These include the degree of chemical definition, e.g. serum-free (SF), animal-derived component free (ADCF) or chemically defined (CD), and the type of medium, e.g. basal media, medium supplements, or full replacement media. In order to specify the cell lines that are adapted to serum-free media, search terms like species, organ, tissue, cell type and disease can be used. All commercially available serum-free media and adapted cell lines currently available from major distributors (e.g. ATCC, ECACC and DMSZ) are included in the database. Despite an extensive search for serum-free media and adapted cell lines, detailed information from certain companies and suppliers is still lacking and is specifically highlighted. It is intended to create a platform for the interactive exchange of information and experience by experts in the field in order to continuously improve and extend the serum-free online database. The database is accessible at http://www.goodcellculture.com/

Keywords: 3Rs, database, fetal bovine serum (FBS), good cell culture practice (GCCP), serum-free cell culture, serum replacement

1 Preface

The supplementation of basal culture media with animal serum of different origins is essential for cell growth, metabolism, and to stimulate proliferation ("mitogenic effect"). The sera used most widely are bovine sera of adult or newborn animals, or of fetal origin. Fetal bovine serum (FBS) is a cocktail of most of the factors required for cell attachment, growth and proliferation and is thus used as an almost universal growth supplement effective for most types of human and animal (including insect) cells. However, the use of animal serum in cell culture also bears a number of disadvantages. These disadvantages can be seen either from

- a theoretical, cell biological point of view, since serum in general is an ill-defined mixture of components in culture media,
- from ethical perspectives in terms of animal protection arguments regarding the harvest and collection of FBS from bovine fetuses and
- in terms of recent concerns about the global supply versus demand of FBS. As a consequence, a number of strategies were developed to reduce or replace the requirement for FBS in cell culture media.
- Furthermore, recent advances in biomedicine, tissue engineering and (adult mesenchymal) stem cell-based therapy demand innovative serum-alternatives of human origin for autologous cell expansion and reimplantation. Also, for the biotechno-

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logical production of human therapeutics, animal-derived components cannot be applied in culture protocols. Therefore, considerable efforts are also directed towards developing animal-free serum alternatives in order to replace FBS in cell culture media.

Exact numbers on the amount of FBS produced for the world market are still unknown. It is estimated that approximately 500,000 litres of FBS are sold per year, which means that more than 1,000,000 bovine fetuses must be harvested (Jochems et al., 2002; Shah, 1999). Recently, serious ethical concerns were raised about donor fetus welfare in the harvest, production and processing of FBS (Shailer and Corrin, 1999; van der Valk et al., 2004). The concerns focused mainly on the current methods of collecting FBS and the suffering of animals, in particular bovine fetuses.

These aspects were the topic of a workshop entitled "Towards Better *In Vitro* Methods: The Replacement of Fetal Bovine Serum". The workshop participants, experts in the field of (fetal) pain and awareness and of serum-free *in vitro* methods, put together a comprehensive workshop report (van der Valk et al., 2004). This workshop, which was held in Utrecht, The Netherlands, was generously sponsored by the Netherlands Organization for Health Research and Development (ZonMw), the Swedish National Board for Laboratory Animals (CFN), and the European Society for Toxicology In Vitro (ESTIV). A follow-up workshop on the "Development of Serum-free Media" was recently held in Copenhagen, Denmark, organised by the Dutch-Belgian Society for In Vitro Methods (INVITROM), the European Society for Toxicology In Vitro (ESTIV) and the Danish In Vitro Toxicology Network and was supported by the Doerenkamp-Zbinden Foundation. First results and recommendations of this workshop were presented at the Joint Autumn Meeting of INVITROM and IVTIP in Antwerp, Belgium (van der Valk et al., 2009).

In recent years, a number of national platforms for alternatives to animal testing, e.g. the Austrian Center for Alternatives and Complementary Methods to Animal Testing (zet, Linz, Austria; http://www.zet.or.at), the Swiss Animal-free Research (formerly FFVFF, Zurich, Switzerland; http://www.animalfree-research.org) and Focus on Alternatives, which comprises several British animal welfare organisations (http://www.focusonalternatives.org.uk), initiated programmes and research projects to discuss alternatives to the use of FBS in cell and tissue culture.

As a major goal of these initiatives, intensive efforts will be undertaken to decrease the global demands for FBS and thus to reduce the number of bovine fetuses harvested.

Therefore, in terms of the 3Rs principle – Reduce, Refine, Replace (Balls et al., 1995) – and thus to avoid/reduce harvesting of FBS from bovine fetuses, researchers should turn to other options for cell and tissue culture, e.g. alternatives to the use of FBS, like serum-free cell culture (Gstraunthaler, 2003), the replacement of FBS by the use of serum-substitutes (Rauch et al., 2008, 2009) or the establishment of databases for serum-free culture media applications (Falkner et al., 2006), in order to provide information resources and to speed up the search for commercially available serum-free media formulations, respectively. These efforts were recently included in a statement by ESAC, ECVAM's Scientific Advisory Committee, on the use of FBS and other animal-derived supplements, which was endorsed at the 28th ESAC Meeting in May 2008[1].

2 Introduction

Cell and tissue culture, i.e. the propagation and cultivation of cells *in vitro* (for definitions see Schaeffer, 1990), has become an indispensable tool in basic cell and molecular biology research, applied biotechnology, and *in vitro* alternatives (Lindl und Gstraunthaler, 2008; McKeehan et al., 1990). Cultured cells form the biological basis of most alternative methods (Hartung, 2007). The use of cell culture is increasing exponentially, and new *in vitro* alternatives are constantly being developed and applied. The propagation and expansion of human cells in tissue engineering (Minuth et al., 1998; Risbud and Sittinger, 2002; Stock and Vacanti, 2001), and in (adult) stem cell technology and cell based therapy has gained increasing importance (Bianco and Robey, 2001; Czyz et al., 2003; Eridani et al., 2004; Tsonis, 2002; Zandstra and Nagy, 2001). In addition, during the past decade, invertebrate cell and tissue culture has become a prominent tool in biotechnological applications, since cultured insect cells are widely used as eukaryotic *in vitro* expression systems using baculovirus expression vectors (Fraser, 1992; Jarvis, 1991; Kost and Condreay, 1999, 2002; Kost et al., 2005; McCarroll and King, 1997).

2.1 The basics in cell and tissue culture

For successful growth, maintenance and expression of differentiated metabolic functions of human or animal cells *in vitro*, either primary cultures or continuous cell lines, appropriate culture conditions are required that mimic the physiological conditions *in vivo et situ* with respect to temperature, pH, osmolarity and oxygen supply (Davis, 2002; Masters, 2000). The

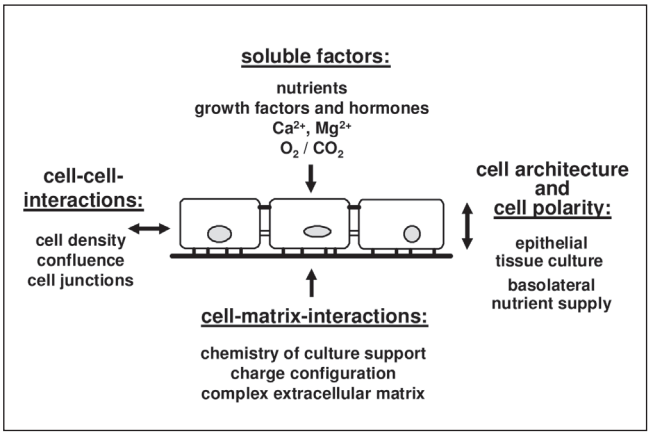

Fig. 1: Parameters controlling growth, proliferation and expression of differentiated functions of cultured cells *in vitro* (Lindl and Gstraunthaler, 2008).

[1] ESAC statement on the use of FCS and other animal-derived supplements, May 2008; http://ecvam.jrc.ec.europa.eu/index.htm

microenvironment of a cell has therefore to be provided by the tissue culture conditions (Fig. 1): by the culture substratum, i.e. the appropriate culture dish or specifically coated surfaces that allow attachment and spreading of cells, and by the culture medium, which provides all types of soluble molecules – nutrients and salts, hormones and growth factors, buffering and oxygen supply (Davis, 2002; Freshney, 2005; Lindl und Gstraunthaler, 2008; Masters, 2000).

From this perspective, the culture medium is by far one of the most important single factors in cell and tissue culture. The culture medium must supply all essential nutrients for cell metabolism, growth and proliferation (Bettger and McKeehan, 1986; Butler and Jenkins, 1989; Ham and McKeehan, 1979; Levintow and Eagle, 1961; Nardone, 1987). These include biosynthetic precursors for cell anabolism, catabolic substrates for energy metabolism, vitamins and trace elements, whose function is primarily catalytic, and bulk inorganic ions (electrolytes), whose functions are both catalytic and physiological, e.g. to maintain culture medium pH and osmolarity within acceptable limits. In addition, animal serum is frequently added to chemically defined basal media.

2.2 The role of serum in cell culture media

Animal serum is an extremely complex mixture of a large number of constituents, low and high molecular weight biomolecules, with different, physiologically balanced growth-promoting and growth-inhibiting activities. The sera most widely used are bovine sera of adult or newborn animals, or of fetal origin (FBS). Modern cell biology and biochemistry allowed the identification of serum (growth) factors involved in *in vivo*

Tab. 1: Typical components (constituents) of serum

Protein Components:	
Serum proteins	Albumin Globulins (e.g. Immunglobulins, IgG) α1-Antitrypsin (Protease Inhibitor) α2-Macroglobulin (Protease Inhibitor)
Transport proteins	Transferrin Transcortin α1-Lipoprotein β1-Lipoprotein
Attachment and Spreading Factors	Fibronectin Laminin Serum Spreading Factor
Enzymes	Lactate Dehydrogenase Alkaline Phosphatase γ-Glutamyl Transferase Alanine Aminotransferase (ALT/GPT) Aspartate Aminotransferase (AST/GOT)
Hormones	Insulin Glucagon Corticosteroids Vasopressin Thyroid Hormones Parathyroid Hormone Growth Hormone Pituitary Glandotropic Factors Prostaglandins
Growth Factors and Cytokines	Epidermal Growth Factor (EGF) Fibroblast Growth Factor (FGF) Nerve Growth Factor (NGF) Endothelial Cell Growth Factor (ECGF) Platelet-derived Growth Factor (PDGF) Insulin-like Growth Factors (IGFs) Interleukins Interferons Transforming Growth Factors (TGFs)
Fatty Acids and Lipids	Free and Protein-bound Fatty Acids Triglycerides Phospholipids Cholesterol Ethanolamine Phosphatidylethanolamine
Vitamins and Trace Elements	Retinol/Retinoic Acid (Vitamin A) Vitamin B-Group: Thiamine Riboflavin Pyridoxine/Pyridoxalphosphate Cobalamin Folic Acid Niacinamide/Nicotinic Acid Panthotenic Acid Biotin Ascorbic Acid (Vitamin C) α-Tocopherol (Vitamin E) Selenium, Iron, Zinc, and Cu, Co, Cr, I, F, Mn, Mo, V, Ni, Sn
Carbohydrates	Glucose Galactose Fructose Mannose Ribose Glycolytic Metabolites
Nonprotein Nitrogens	Urea Purines/Pyrimidines Polyamines Creatinine Amino Acids

processes, like cell proliferation and tissue repair (e.g. wound healing; Werner and Grose, 2003), and cell maturation and differentiation (e.g. embryonic maturation, stem cell lineage, epithelial differentiation, etc.) (Tab. 1).

The major functions of serum in culture media (Tab. 2) are to provide (i) hormonal factors stimulating cell growth and proliferation and promoting differentiated functions, (ii) transport proteins carrying hormones (e.g. transcortin), minerals and trace elements (e.g. transferrin) and lipids (e.g. lipoproteins), (iii) attachment and spreading factors, acting as germination points for cell attachment and (iv) stabilising and detoxifying factors needed to maintain pH or to inhibit proteases either directly, such as α-antitrypsin or α2-macroglobulin, or indirectly, by acting as an unspecific sink for proteases and other (toxic) molecules.

It is a well known fact that natural clotted serum is more effective than plasma in stimulating cell proliferation. This appears to be due to the release of certain polypeptides from activated platelets during the clotting process (Balk et al., 1981; Gospodarowicz and Ill, 1980).

In recent decades, a number of growth factors, hormones, transport proteins, co-factors, essential minerals and trace elements have been identified, all of which drive specific gene expression, initiate and control the cell cycle and thus cell division, and programme specific cell differentiation (Hill and Treisman, 1995). We know today, for example, that a specific transcriptional activation programme is required to initiate cell growth and proliferation *in vitro*, e.g. the specific activation of mitogen-activated protein (MAP) kinases, like the extracellular signal-regulated kinase (ERK) cascade (Denhart, 1996; Kyriakis and Avruch, 2001; Widmann et al., 1999), by epidermal growth factor (EGF) (Carpenter and Cohen, 1979; Cohen, 1987) or platelet-derived growth factor (PDGF) (Heldin et al., 1985; Ross et al., 1986). Other polypeptide growth factors, which are present in animal serum and activate cultured cells with varying degrees of specificity, are fibroblast growth factor (FGF) (Gospodarowicz et al., 1987), nerve growth factor (NGF) (Levi-Montalcini, 1987), endothelial growth factor, or the insulin-like growth factors IGF-1 and IGF-2 (Deuel, 1987) (Tab. 1).

Because of its rich content of growth factors and its low γ-globulin content, FBS has been adopted as the standard supplement of cell culture media. In most cases, FBS is used at concentrations of 10% (v/v), although this may vary for specific applications (e.g. low serum media). Other advantages in the use of FBS include the following: (i) FBS is a cocktail of most of the factors required for cell proliferation and maintenance (see above); (ii) it is thus an almost universal growth supplement effective with most types of human and animal (including insect) cells. (iii) The use of serum-supplemented medium therefore reduces the need to spend time and effort developing a specific, optimised medium formulation for each cell type.

Tab. 2: The role of serum in cell culture media

Role of Serum in Cell Culture Media
Serum provides
> growth factors and hormones
> binding and transport proteins
> attachment and spreading factors
> additional amino acids, vitamins and trace elements
> fatty acids and lipids
> protease-inhibitors
> "detoxification" (due to binding and inactivation)
> (colloid)osmotic pressure
> reduction of shear stress

The use of animal serum in cell culture also bears a number of disadvantages. These disadvantages can be seen either from a theoretical, cell biological point of view, from ethical and animal welfare perspectives, and/or in terms of global supply versus demand of FBS.

A) Cell biological perspective

From a cell biological perspective, the disadvantages of supplementation of culture media with FBS are manifold: (i) Serum is an ill-defined medium supplement, and thus an ambiguous factor in cell culture (Bjare, 1992; Gstraunthaler, 2003), (ii) serum batches display quantitative and qualitative variations in their composition and thus introduce a serious lot-to-lot variability (Price and Gregory, 1982), (iii) serum may contain different amounts of endotoxins, haemoglobin, and other adverse factors, and (iv) serum can be a potential source of microbial contaminants, such as fungi, bacteria, mycoplasma, viruses or prions (Dormont, 1999; Eliot, 1999; Wessman and Levings, 1999). Thus, serum introduces several unknown variables into the tissue culture system (Even et al., 2006; Lindl und Gstraunthaler, 2008; Stoll and Spector, 1984). In addition, serum-supplemented media may be unable to support the growth of specific cell types, and in primary cultures of highly differentiated epithelial cells, serum may be unable to prevent the overgrowth of the culture by fibroblasts (Taub, 1990).

B) Ethical concerns and animal welfare issues

The amount of FBS produced for the world market is estimated to approximate 500,000 litres per year. As a consequence, more than 1,000,000 bovine fetuses have to be harvested, and these numbers are expected to increase annually (Jochems et al., 2002; Shah, 1999). Recently, serious ethical concerns were raised with regard to the welfare of the donor fetuses in the harvest, production and processing of FBS (Shailer and Corrin, 1999; van der Valk et al., 2004). The concerns were mainly focused on the current modes of collecting FBS that may cause suffering to the animals, in particular to fetuses. In brief, the bovine fetuses from which blood is drawn for FBS production are obtained from pregnant cows sent to slaughter. In huge meat cattle herds, bulls and cows roam freely together, and as a result many cows are pregnant at the time of slaughter. When a pregnant cow is discovered at the routine slaughterline, the fetus is separated at the abattoir, and fetal blood is collected under aseptic conditions. This is usually performed by puncturing the beating heart of the unanaesthetised fetus using large diameter needles. Alternatively, fetal blood may be harvested by means of umbilical vein puncture or puncture of the jugular vein (Jo-

chems et al., 2002). Thus, fetal blood collection involves significant manipulation of the fetus.

Opinions on the issue of fetal awareness are still divided (van der Valk et al., 2004). Nociperception is developed in bovine fetuses after 5 months of gestation. Thus, the most effective way to collect raw serum involving the least possible harm to fetuses is to ensure that they are not conscious (Mellor and Gregory, 2003). When bovine fetuses are exposed after slaughter of the dam, they can suffer only if they inflate their lungs with air and increase their blood oxygen to levels compatible with awareness. Recent research on electrical brain activity of bovine fetuses indicates that, under normal circumstances, they do not exhibit consciousness before birth (Mellor et al., 2009a, 2009b).

However, despite tight animal welfare regulations, the specific situation at the local production sites may not always guarantee best practice in treating the fetuses during blood collection. The most rigorous legislation and policies in this respect are established in New Zealand (Shailer and Corrin, 1999). Blood, sera and tissues from slaughtered animals must be collected at licensed abattoirs or slaughterhouses with an accompanying veterinary certificate stating collection details.

C) Global supply versus demand of FBS

The availability of FBS has changed dramatically over the past few years (Fujimoto, 2002). Therefore, all efforts and attempts should be undertaken to overcome the predicted ever tightening supply of FBS. In the case of FBS, supply versus demand models do not follow typical economic principles. Normally, supply can be adjusted to meet the demand. However, in case of FBS, supply and demand operate independently of each other.

Fetal bovine serum is a by-product of the beef packing industry. Thus, the supply is dictated by many factors, including beef consumption, dairy product consumption, feed prices, environmental factors, such as drought, cattle import and export, governmental farm policies (Shailer and Corrin, 1999) and the outbreak of diseases (foot and mouth disease, BSE) (Dormont, 1999; Asher, 1999). Worldwide beef consumption continues to decline as people switch from red meat to other meat sources such as poultry. Additionally, the efficiency of beef production has improved through genetics and better nutrition, resulting in an increased meat production with fewer animals. All this could lead to a slow but steady decline in the global supply of FBS in the future.

According to recent reports (Häusl, 2008), the situation has been further aggravated. Under the headline "Fetal Bovine Serum running short" it is reported that EU legislators imposed an import stop for fresh meat from Brazil to the EU. In 2007, approximately 70% of the FBS used in European medical research came from Brazil. After the import ban on Brazilian meat, livestock slaughter rates fell by 18%. This decline in the number of slaughtered cattle led to a disproportional decrease in FBS production. Ironically, the import ban on Brazilian meat by the EU did not involve the import of Brazilian FBS.

In contrast, the demand for FBS has increased worldwide at a slow but steady rate, primarily because of an increased protein-based drug production by the biopharmaceutical industry. Vaccine production also accounts for a significant percentage of the FBS demand. But also the use of FBS in research laboratories, in biomedicine and tissue engineering continuously increases.

From the disadvantages in the use of FBS in culture media, the advantages and benefits of serum replacement are thus easily deducible (Tab. 3).

2.3 Alternatives to fetal bovine serum in cell and tissue culture

In light of the above mentioned disadvantages and concerns, a number of methods for reducing the requirements for FBS in culture media as well as alternative animal serum substitutions were recently described (Jayme et al., 1988). The latter include the substitution by newborn or adult bovine sera, or the use of donor sera from other animal species (horse, pig, goat, etc.). Other animal-derived alternatives are tissue extracts (e.g. pituitary extracts, chicken embryo extracts), ocular fluid (Filipic et al., 2002), bovine milk fractions, or bovine colostrum (Belford et al., 1995; Klagsbrun, 1980; Pakkanen and Neutra, 1994; Steimer et al., 1981). Most recently, protein fractions from plant extracts, called vegetal serum (Pazos et al., 2004), and human platelet lysates (Rauch et al., 2008, 2009) were successfully introduced to grow and maintain epithelial cells in culture. One problem in the use of alternative complexes that should be kept

Tab. 3: The benefits of serum replacement

Benefits of Serum Replacement
> cell biological aspects: - chemically defined and controlled culture conditions *in vitro* - reduced variability in qualitative and quantitative culture medium composition - reduced risks of microbial contamination (mycoplasma, viruses, prions) - advantages in the isolation of cell culture products (down-stream processing)
> ethical aspects: - to reduce or completely avoid the suffering of fetuses and animals
> independence of commercial supply

in mind is to provide a continuous availability and reliable commercial supply. The vegetal serum alternative, called Prolifix, is, for example, now no longer marketed (Pazos et al., 2004; van der Valk et al., 2004). Human platelet lysates can be isolated from outdated donor thrombocyte concentrates as described (Rauch et al., 2008, 2009).

Additional methods of choice for reducing the requirement of FBS include (i) the optimisation of existing culture media, (ii) the use of reduced serum media, (iii) the design of chemically defined, serum-free media (Barnes and Sato, 1980a, 1980b; Barnes et al., 1987; Bjare, 1992; Defrancesco, 1998; Froud, 1999; Gstraunthaler, 2003; Jayme et al., 1988; Taub, 1990; Zimmermann et al., 2000) or (iv) the establishment of databases for serum-free culture media applications (Falkner et al., 2006) in order to provide well focused information to ease the use of and the switch to serum-free cell and tissue culture, respectively.

In recent years, the commercial supply of serum-free media has increased substantially. The screening of catalogues of vendors and companies, however, is time-consuming and often does not provide satisfactory results. Moreover, an almost complete list of relevant suppliers may not be available in all laboratories.

3 The serum–free media interactive online database

In the present database for serum-free cell culture, accessible at http://www.goodcellculture.com/, all commercially available serum-free media and medium supplements have been compiled in a searchable matrix. Furthermore, cell lines, hybridomas and primary cells distributed by the main cell banks (ATCC, ECACC and DSMZ) are referenced in order to facilitate the search for serum-free media by selecting the appropriate cell line. All products (media and cells) are specified in categories of species, organs, cell types, diseases and growth properties as described below.

3.1 Structure of the database

The internal structure of the database is depicted schematically in Figure 2. Serum-free media are associated with all types of cells, either primary cultures or continuous cell lines, for which serum-free culture formulations are available through defined attributes: species, organ, cell type and disease. In addition, culture media are categorised into *growth media* and *application media*, and into the *chemical degree* of the medium formulation.

A complete description or dataset for each culture medium or cell consists of species, organ, cell type, disease and growth property. In order to obtain qualitatively and quantitatively satisfactory results, the information is standardised and categorised before being made available online. Species were divided according to ITIS (Integrated Taxonomic Information System) and diseases according to WHO ICD10 (International Classification of Diseases). For organs and cell types a system was generated to suit the needs of comfortable access.

Cells and media are compatible when a complete match of a dataset is accomplished. "Lack of Information" is treated as non-existing criteria and included in the search as omnipotent aside from diseases. Because of the vast numbers of products related to diseases (e.g. tumour cells or lymphoma cells, and of media suitable for these cells), a lack of information on the disease status is treated as not diseased.

If "Lack of Information" appears as the result of a given search, it is up to the user to decide whether the result is suitable for his or her purpose.

In addition to the description of physiological and taxonomical categories, serum-free media are associated with all types of cells, either primary cultures or continuous cell lines, and categorised into *growth media* and *application media*.

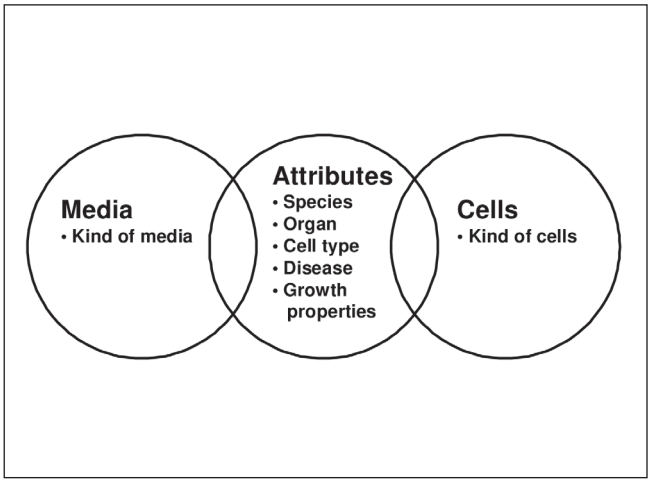

Fig. 2: Structure of the database.
Media are associated with cells through defined attributes (species, organ, cell type, disease and growth properties).

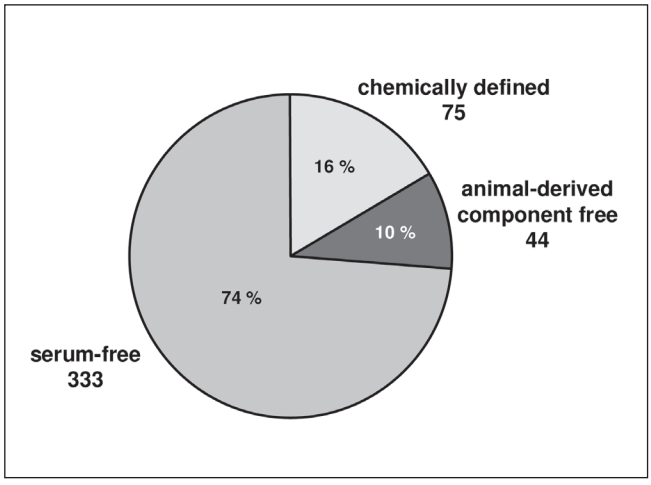

Fig. 3: Cell culture media contained in the database.
Media, and thus search terms, are grouped into chemically defined (CD), animal-derived component free (ADCF), and serum-free (SF).

Tab. 4: Content of the database

A. Media listed according to chemical degree (Fig. 3)

Chemically defined media	75 (16%)
Animal-derived component free media	44 (10%)
Serum-free media	333 (74%)
total	**452**

B. Kind of media (Fig. 4)

Replacement media	Chemically defined	56	
	Animal-derived component free	30	
	Serum-free	171	257 (57%)
Basal media	Chemically defined	6	
	Animal-derived component free	3	
	Serum-free	81	90 (20%)
Supplements	Chemically defined	10	
	Animal-derived component free	1	
	Serum-free	39	50 (11%)
Application media	Chemically defined	3	
	Animal-derived component free	10	
	Serum-free	42	55 (12%)
total			**452**

C. Kind of cells: cell lines, hybridoma and primary cultures

Continuous cell lines	3694 (77%)
Hybridoma lines	1108 (23%)
Primary cultures	15 (0.003%)
total	**4817**

D. Cell lines according to tissue origin or cell type

Lymphoid lines	1739 (76%)
Kidney cells	268 (11%)
Hepatocytes	80 (4%)
Neuronal cells	189 (9%)
total	**2276**

The category *growth media* describes a medium's primary ability to stimulate growth or to permit culturing. Growth media are declared as replacement media, basal media or supplements. The category *application media* describes the primary use of the media, e.g. for specific metabolic functions, like transgenic protein production, cell differentiation or as simple wash solution, transport medium, etc.

The main purpose of the database is to facilitate the search and use of serum-free media, respectively. The search term *chemical degree* describes the contents/formulation of media or the status of cells adapted to specific media. The following attributes are available:

– CD (chemically defined): Chemically defined media do not contain proteins, hydrolysates or any other components of unknown composition. Highly purified hormones or growth factors added can be of either animal or plant origin, or are supplemented as recombinant products (see: animal-derived component free media).
– ADCF (animal-derived component free): Media containing no components of animal origin. These media are not necessarily chemically defined (e.g. when they contain bacterial or yeast hydrolysates or plant extracts).
– SF (serum-free): Serum-free media do not require supplementation with serum but may contain discrete protein fractions (e.g. animal tissue or plant extracts) and are thus regarded as chemically undefined (see: chemically defined media).

The search term *cells* is categorised at present into cell lines, hybridomas and primary cultures. If necessary, additional categories can be added.

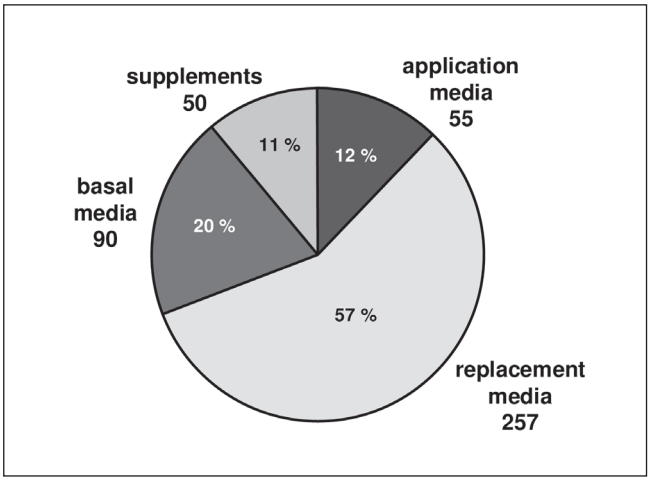

Fig. 4: The four major kinds of culture media listed in the database:
replacement media, basal media, medium supplements and single-purpose application media.

3.2 Content of the database

The actual content of the database is summarised in Table 4. At present, the "good cell culture" database contains more than 450 serum-free media. These are grouped into 75 chemically defined media, 44 animal-derived component-free media and 333 serum-free media (Fig. 3). 257 Serum-free media are full replacement media that do not need any supplementation. 90 media are basal media with 50 supplements available. In addition, 55 single purpose application media that do not overlap with other media are contained in the database at present (Fig. 4). The absolute num-

bers of media and cells, respectively, reflect the current version of the database and may change as new media and/or adapted cell lines are marketed and the database is updated accordingly. The amount of serum-free media, in respect to animal-derived component-free and chemically defined media, exceeds chemically defined media by 4 to 1 and animal-derived component free media by 7.5 to 1, respectively.

Furthermore, 3694 continuous cell lines and 1108 hybridoma lines are contained in the database[2]. Most cell lines are of primate/human origin, followed by rodent cell lines. Hybridomas are mainly of rodent origin.

The following information about media and cells is accessible:

– Chemical Degree – contents of media or the status of cells adapted to specific media
– Growth properties – adherent, suspension or mixed
– Protein free – protein free media or the status of cells adapted to protein free media
– Tested Cells/Media – many media are suitable for specific cells according to companies that can be accessed directly.

3.3 Search modi in the database

In the "good cell culture" database, online searches can be performed for culture media, cells and/or companies/vendors (in alphabetical order). For each medium or cell type, the desired chemical degree can be selected in addition. There is no restriction on switching from media to cells and vice versa at any time. Independent of demand, two search modi can be used to browse as efficiently as possible: *standard search* and *power search*. Each search is performed on a specific interface.

In *standard search*, a simple comparison of attributes is used to identify identical pairs in species, organ, cell type and disease of media and cells, respectively (Fig. 5). A text search leads to refinement of media or cells by using nouns. Due to a lack of synonyms and adjectives, which will be implemented in future refinements of the database, at present only the noun form of organs (e.g. kidney instead of renal, heart instead of cardiac), cell types and diseases, and Latin nouns for species can be searched.

In a second approach (*power search*), criteria such as species, organs, cell types and diseases that are embedded within structure-like datasets as described above, can be selected individually. The unique method of *power searching* is to treat input as category, which means specifying all terms like species, organ, cell group or cell type, disease and growth properties by subjective emphasis. It is up to the user to choose similarity by restricting these terms.

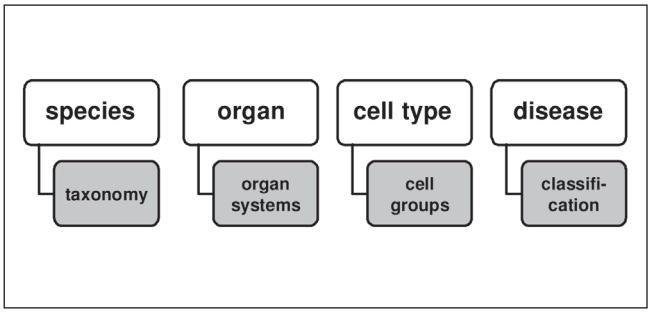

Fig. 5: Correlation between species, organ, cell type and disease as implemented in the database.

4 Future perspectives

The interactive online database is dependent on information provided by companies and scientists. It is therefore essential, to gather more valid data. In future, a scientific forum will be established and company information will be presented in order to maintain an up-to-date interactive database of serum-free media and cell lines.

Questions regarding the database, as well as any comments and information, may be sent to info@goodcellculture.com

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[2] Regarding sum of all cells distributed by ATCC, ECACC and DSMZ. Duplicates regarding distribution are included.

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