mammalian
Cell & Tissue Culture Magazine
Issue 1

History of KSOM
A Single Medium for Embryo Culture
by John D. Biggers, DSc, PhD

A Comparison of GPS and Standard Dishes for Embryo Culture: Effects on Set-Up and Observation Times and Embryo Development
by D. Rieger, T. Schimmel, J. Cohen and M. Cecchi

New Hope for Infertility Therapy: Fabricating Gametes from Stem Cells
by Huai L. Feng, PhD

SSR 2008, 41st Annual Meeting
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Dear Reader,

Welcome to the inaugural issue of mammalian Cell & Tissue Culture Magazine. This magazine is our vehicle for sharing current and relevant cell, tissue and stem cell information at a time when it is so needed and to provide a forum for the presentation of reviews of concepts, techniques, and clinical applications of cell and tissue culture.

mammalian Cell & Tissue Culture Magazine’s content will be articles provided by you the reader, scientifically based information, in an informal and more relaxed venue.

I would like to thank the contributors of this issue, for providing us with several informative and timely articles, as well as our advertisers who took a ‘leap of faith’ to provide us with the resources to make this possible.

I invite you to submit articles that are relevant to mammalian cell and tissue culture, and aimed toward an audience of scientists, clinicians, and other interested people. We look forward to your contributions to future issues.

Don Rieger, PhD

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History of KSOM
A Single Medium for Embryo Culture

Since 1993 a family of media for the culture of preimplantation embryos has been designed that has acquired the generic name KSOM (Biggers, 2002). The initial work on the design of these media was done as part of the National Cooperative Program on Non-Human In Vitro Fertilization and Preimplantation Development, sponsored by the NICHD between 1986 and 1996, with the objective of improving embryo culture. Since the original formulation of a medium called SOM (Lawitts and Biggers, 1991), four modifications have been published:
- KSOM (Lawitts and Biggers, 1993)
- mKSOM (now called KSOMg) (Summers et al., 1995)
- KSOMAA (Ho et al., 1995, Biggers et al., 2000)
- KSOMgAA (Biggers and McGinnis, 2000).

In the early days of preimplantation mouse embryo culture, heralded by the pioneer paper of Whitten (1956), a major problem had been an arrest of development at the two-cell stage, called the two-cell block. Over the years several seemingly unrelated ways of overcoming this block were described (review: Biggers, 1993), which led to the notion that the media being used were unbalanced and hence not optimal. Optimizing culture media in general is a complex matter because of the need to take account of the possible interaction of effects between its components (Biggers et al., 1957). This is a general problem in many areas and was first studied in the optimization of industrial processes such as the manufacture of chemicals. One solution that was introduced was called sequential simplex optimization (see sidebar). Our research involved determining the proportion of embryos that developed beyond the two-cell stage as the concentrations of constituents in a start medium were simultaneously varied, according to the rules determined by the optimization protocol. After twenty iterations, taking about two years, a medium called SOM was developed which overcame the two-cell block (Lawitts and Biggers, 1991). Soon after, the composition of the medium was slightly modified to formulate KSOM (Lawitts and Biggers, 1993), based on measurements of the intracellular ionic composition of the blastomeres determined by electron probe microanalysis. An unexpected bonus of this research was the fact that KSOM supported a high yield of mouse blastocysts from zygotes that were capable of developing into fetuses after transfer into the uterus of surrogate mothers (Erbach et al., 1995). The medium was greatly improved when Ho et al. (1995) supplemented KSOM with 19 natural amino acids (glutamine was already in the medium). In another study very high yields of expanded blastocysts were obtained containing nearly double the numbers of inner cell mass and trophectoderm cells compared to KSOM alone (Biggers et al. (2000a). This medium is called KSOMAA.

A variant of KSOM (mKSOM) was introduced to support IVF in the mouse. The original version of KSOM contains a low concentration of glucose (0.2mmol/l), which does not support the viability of sperm and therefore cannot be used for IVF. When the concentration of glucose in KSOM was raised to that found in blood (5.56mmol/l), IVF in the mouse was successful (Summers et al., 1995). This result was particularly surprising since this concentration of glucose did not prevent the subsequent cleavage divisions, contrary to the widespread dogma asserting that glucose
A medium developed by Sequential Simplex Optimization

inhibits the development of the preimplantation embryo. Subsequent studies have confirmed that glucose in a concentration as high as 5.56mmol/l in KSOMAA does not inhibit the early cleavage divisions of the mouse embryo (Biggers et al., 2000b). This medium is now called KSOMg.

The inclusion of glutamine in media used for the culture of cell lines has always been of concern because its instability leads to the accumulation of ammonium in the media. Gardner and Lane (1993) alerted the IVF community to the putative dangers of including glutamine in embryo culture media by reporting a disturbing incidence of exencephaly in mice whose preimplantation embryos had been cultured in a medium containing the compound. More recent studies, by two independent laboratories, have failed to detect any incidence of gross abnormal development in fetuses and newborn mice whose preimplantation embryos have been cultured in KSOM-type media. Possible reasons for the discrepancies have been discussed by Biggers et al. (2004a). Nevertheless, concern about the potential adverse effects of glutamine in media can be alleviated by replacing glutamine with a dipeptide containing glutamine. Alanylglutamine is commonly used for this purpose. However, glycylglutamine may be preferable since there is evidence that it favors the development of the ICM (Biggers et al., 2004b). Glycylglutamine is therefore included in all currently used variants of KSOM in our laboratory.

It has become almost universal to use a two-step protocol when it is desired to culture human zygotes to the blastocyst stage following the early recommendations of Gardner (1998). The protocol involves the sequential culture of the embryos in media of different chemical composition. There are several pairs of such media available commercially. The justification for changing the medium in the middle of the culture period is either to remove putative toxic substances that have accumulated, or to imitate the natural environment which changes as the embryos pass from the oviduct into the uterus. Unfortunately there have been few experimental investigations to verify the need for a two-step protocol. A paper that has been submitted for publication describes results which show that there is no gain in renewing KSOMg during the culture of the mouse preimplantation embryo. The studies of Biggers and Racowsky (2002) using KSOMAA and Macklon et al. (2002) using the so-called “Rotterdam” medium, failed to show an advantage of the two-step procedure, for the culture of human preimplantation embryos, suggesting that a more thorough examination of the practical advantage of two-step procedures should be undertaken.

The ultimate medical objective of treatment for infertility is the production of normal healthy babies. The techniques used to evaluate treatment protocols has involved such parameters as the rates of embryo development before transfer, and the rates of biochemical pregnancies and delivery rates. There has always been a lingering concern that some constituents of media may have deleterious effects (review: Summers and Biggers, 2003), particularly epigenetic effects that may be passed on to later generations (review: Johnson, 2005). Advances in molecular genetics are beginning to open up studies on these questions. For example, Rinaudo and Schultz (2004) have clearly shown that media can effect the expression of genes in mouse blastocysts cultured from the zygote to the blastocyst stage; 114 genes were mis-expressed in Whitten’s medium and only 29 genes in KSOMAA. Whether this difference can account for the better development in KSOMAA needs detailed analysis.

References
Imagine a medium with only two components. There are an infinite number of media possible, each defined by the paired concentrations of each of the two components. Imagine also that we know the response of an embryo when cultured in each medium. Then the totality of responses can be represented by a concentration-response surface (Figure 1). We assume that the maximum response is the optimum response. How do we locate the maximum of the concentration-response surface? With only two components we can choose a set a media that form a grid over the surface. The grid defines a set of media to be compared in a factorial experiment. From the results of this experiment a concentration response surface can be fitted and the maximum located.

When media contain more than two components, the concentration surface will be multi-dimensional. In producing SOM we optimized 10 media so the response surface was modeled in 11-dimensional space. Locating a maximum in this space is logistically impossible using a set of factorially arranged media since a prodigious number of media combinations would be required. An alternative approach is to climb sequentially the surface. Sequential simplex optimization is one of several algorithms that allow this climb to be made.

Data already obtained is used to locate a point of the putative hill; this point defines the composition of a START medium (Figure 1). If the medium consists of two components only, a set of three media are chosen in the neighborhood of the START medium, defined by the loci of three vertices of a triangle (a triangle is a simplex in two-dimensional space). The responses to these media, (in this case passage through the two-cell block) are observed experimentally and the medium that gives the worst response is identified. A new medium is then determined from these results which is higher up the hill, generating a new simplex consisting of the two non-rejected original media and the new medium. The new set of three media are then compared and the experimental procedure repeated. By repeating this procedure the hill will be climbed and its top reached. In the case of 10 media, 11 media are compared at each step. A detailed description of the procedure can be found in the website http://www.multisimplex.com/ algorithms.htm.
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Introduction
In recent years, the field of reproductive medicine has been revolutionized by recent studies demonstrating that mouse and human embryonic stem (ES) cells are capable of forming primordial germ cells (PGCs) *in vitro* (Kehler *et al.*, 2005). The mouse germ cells appear to be capable of undergoing meiosis and forming both male and female gametes *in vitro*. Although the full function of these ES-derived germ cells and gametes remains to be demonstrated, these findings give new hope for infertility therapy and novel approaches to regenerative medicine.

Male Gamete Formation In Vivo
Approximately 50% of human infertility is attributable to male defects including oligo-, astheno-, teratospermia and azoospermia (Sigman *et al.*, 1997). Treatments for male infertility are limited and a range of in-vitro fertilization (IVF) techniques are used to circumvent, rather than treat, male infertility problems. Intracytoplasmic sperm injection (ICSI) is most frequently used in cases of male infertility. With ICSI, it is possible to use individual sperm or germ cells recovered from various maturation stages for fertilization. Intracytoplasmic sperm injection (ICSI) is most frequently used in cases of male infertility. With ICSI, it is possible to use individual sperm or germ cells recovered from various maturation stages for fertilization. To develop true therapies, for example to restore spermatogenesis by using stem cell transplantation, will require a deeper understanding of spermatogenesis, which might be provided by studies in the mouse (Cooke *et al.*, 2002). The transplantation of donor mouse male germ stem cells into recipient seminiferous tubules resulted in the formation of spermatozoa morphologically characteristic of the donor species, and production of offspring from donor germline stem cells (Kanatsu-Shinohara, *et al.*, 2003). In the testis, Sertoli cells interact with the germ cells and defects in the Sertoli cells compromise spermatogenesis, leading to male infertility. However, it has not been possible to restore spermatogenesis from endogenous stem cells in infertile testis with environmental defects. This defect in the male germline microenvironment can be corrected by Sertoli cell transplantation and result in normal offspring (Kanatsu-Shinohara *et al.*, 2005), which indicates a promising opportunity to develop a new strategy for the treatment of human male infertility.

A series of investigations failed to establish a complete human spermatogenic line in the testes of mutant aspermatic (W/Wv) mice or severe combined immunodeficient (SCID) mice. The SCID mice received human spermatogenic germ cells obtained from testicular biopsies of non-obstructed or obstructed azoospermic men undergoing infertility treatment. However, sections from the recipient testes examined up to 150 days after transplantation showed recipient seminiferous tubules lined mainly with recipient Sertoli cells, but human germ cells were not found. The authors speculated that the donor germ cells were unable to survive and colonize the mouse testes due to non-compatible cellular interactions and immunological rejection resulting from interspecies differences (Reis *et al.*, 2000).

Therefore, new therapeutic approaches can be envisaged for human spermatogenic failure. A germ stem cell or Sertoli cell transplantation procedure combined with developments in freezing, long-term culturing or enriching germ cell populations will be particularly useful for restoring fertility to those who become infertile after malignancy therapy by chemicals or radiation. Although no method for fertility protection is currently available for prepubertal boys who do not have sperm, stem cell transplantation will provide a method to recover their...
fertility, since spermatogenesis occurs by transplantation of spermatogonial stem cells even from immature donors (Shinohara et al., 2001). Assay and cell sorting techniques must be developed to minimize the risk of transferring malignant cells back into the patient (Sofikitis et al., 2003).

**Male Gamete Formation In Vitro**

Recent development of spermatogonial stem cell culture techniques enables in-vitro expansion of stem cells from a small biopsy sample for autogous transplantation (Kanatsu-Shinohara et al., 2005). As the method to culture Sertoli cells develops, it will be possible to correct the defect in Sertoli cells to be used for autologous transplantation in vitro as well. A more recent study indicated that mouse embryonic stem cells can form germ cells in vitro (Toyooka et al., 2003). Furthermore, mouse embryonic stem cells are capable of forming primordial germ cells and haploid male gametes (spermatocytes), which, when injected into oocytes, restore the somatic diploid chromosome complement and develop into blastocysts (Geljsen et al., 2004; Surani, 2004) (Figure 1). The possibility to derive germ cells and male gamete from embryonic stem (ES) cells could have a very significant impact on assisted reproduction, enabling male infertile individuals to have their own genetic children.

---

**Follicular Development and Female Gamete Formation In Vivo and In Vitro**

A basic doctrine of reproductive biology is that most mammalian females lose the capacity for germ-cell renewal during fetal life, such that a fixed reserve of germ cells (oocytes) enclosed within follicles is endowed at birth (Franchi et al., 1962). A recent study indicated that juvenile and adult mouse ovaries possess mitotically active germ cells that, based on rates of oocyte degeneration (atresia) and clearance, are needed to continuously replenish the follicle pool. Consistent with this, treatment of prepubertal female mice with the mitotic germ-cell toxicant busulphan eliminates the primordial follicle reserve by early adulthood, without inducing atresia. Furthermore, wild-type ovaries grafted into transgenic female mice with ubiquitous expression of green fluorescent protein (GFP) become infiltrated with GFP-positive germ cells that form follicles (Johnson et al., 2004). Collectively, these data establish the existence of proliferative germ cells that sustain oocyte and follicle production in the postnatal mammalian ovary.

Earlier and recent in-vivo and in-vitro studies have shown that mouse embryonic germ cells differentiate from a somatic lineage, and that adult ovaries of prosimian primates and mice possess mitotically active germ cells of uncertain origin (Bulkovsky et al., 2004). Bulkovsky et al. (2004) provided direct evidence that in the human, the components for new primary follicles, primitive granulosa, and germ cells, differentiate sequentially and de novo from the cytokeratin(CK)+ mesenchymal progenitor cells residing in the ovarian tunica albuginea, and that new primary follicles are formed by assembly of oocytes with nests of primitive granulosa cells in the ovarian cortex. The number of newly-formed primary follicles in adult human ovaries appears to be determined by the number of developing nests of primitive granulosa cells supplied by a higher number of available oocytes. Formation of new primary follicles throughout the reproductive period may compensate for the well documented atresia of a significant proportion of the follicular pool (Bulkovsky et al., 2004). These data may contribute to the selection of the best possible oocytes and ensure preservation of the relatively constant number of primary follicles found in human females between 18 and 38 years of life.

Furthermore, research has shown for the fist time that human eggs may develop directly from cultured ovarian surface epithelium (OSE) (sources of ovarian germ stem cells) derived from adult human ovaries that were obtained from five women aged 39 to 52. Oocytes derived from the culture of OSE cells developed in vitro into mature eggs suitable for fertilization and development into an embryo (Bukovsky et al., 2005). The ability to produce mature human eggs from adult ovaries in vitro has several potential applications in human reproduc-
The technique of harvesting cells from the ovarian surface is relatively easy, can be accomplished by a laparoscopy technique, and yields more cells for use for in-vitro fertilization. The ability to develop human eggs from OSE cells may help women with reduced fertility and premature menopause, who lack follicles in their ovaries, to have a better chance of conceiving through in-vitro fertilization. Eventually, frozen OSE cells from younger females may be preserved for later production of fresh eggs. This may prevent the occurrence of fetal genetic alterations, which are often associated with an advanced maternal age. In addition, a colonization of premenopausal ovaries with younger oocyte and granulosa stem cells may establish a new cohort of primary follicles. This may result in a 10- to 12-year delay of the onset of natural menopause. Also, these ovarian stem cells could be used to generate several cell types used in stem cell research, and fertilized eggs produced in this way could produce cells capable of giving rise to embryonic stem cells for use in research and therapeutic applications (Bukovsky et al., 2005).

The results of a study by Johnson et al. (2005) suggest that adult mouse ovaries rapidly generate hundreds of oocytes, despite a small premeiotic germ cell pool. In considering the possibility of an extragonadal source of germ cells, it also showed expression of germline markers in bone marrow (BM). Further, BM transplantation restores oocyte production in wild-type mice sterilized by chemotherapy, as well as in ataxia telangiectasia-mutated gene-deficient mice, which are otherwise incapable of making oocytes. Donor-derived oocytes are also observed in female mice following peripheral blood transplantation. Although the fertilizability and developmental competency of the BM and peripheral blood-derived oocytes remain to be established, their morphology, enclosure within follicles, and expression of germ-cell- and oocyte-specific markers collectively support that these cells are bona fide oocytes. These results identify BM as a potential source of germ cells that could sustain oocyte production in adulthood (Johnson et al., 2005).

Hubner et al. (2003) have shown that mouse ES cells are capable of differentiating into oocytes and forming structures very similar to normal follicle. Release of these oocytes from the surrounding cells led to spontaneous activation and development to the blastocyst stage, thereby demonstrating that these cells are totipotent even in vitro. Future experiments will reveal whether the oocytes that have generated in culture from ES cells can be fertilized, whether they have undergone a gender-specific resetting of the epigenetic marks (imprinting), and whether they can be used as starting material to derive ES cell lines after nuclear transfer (Hubner et al., 2003) (Figure 1). All data suggest that human eggs derived in culture could have an even more exciting use. By following the same procedure, it might be possible to use these eggs to generate ES cells that produce diseased tissues - the adult nuclei for the process being taken from patients with complex diseases such as diabetes. Such ES cells may be repaired or corrected in defect gene, the repaired or health ES cell would provide an unlimited resource, allowing approaches to the treatment of disease that are currently impossible. This might, in turn, lead to new treatments in male and female infertility.

In summary, the possibility of fabricating gametes from stem cells, and generating viable embryos provides hope for the development of new infertility therapies, and may have a significant impact on assisted reproductive medicine in the next decade. Although such therapies may seem like science fiction, they merit international discussion to develop a unified set of medical, ethical, and safety (such as genetic, epigenetic and infection related risks) guidelines that accommodate the moral convictions of patients, physicians, and politicians from diverse social and religious backgrounds (Kehler et al., 2005).

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A Comparison of GPS and Standard Dishes for Embryo Culture: Effects on Set-Up and Observation Times and Embryo Development

Introduction

In standard microdrop culture, embryos are cultured in microdrops of medium on a flat surface. Consequently, the microdrops can flatten, move, or coalesce and the embryos can be difficult to find and examine. In contrast, in GPS culture, embryos are cultured in equally small volumes but, as shown in Figures 1 and 2, the medium is constrained within microwells. The embryos migrate to the center of the microwells and the microwell bottoms are parafocal, so that the embryos are easy to locate and examine. The objective of this experiment was to determine whether the design of the GPS dish would reduce the time required for the set-up of culture and daily examination of mouse embryos.

Materials and Methods

The medium used for all cultures was KSOM + 10 mg/ml BSA. On Day 0, 13 ml of oil was placed into each of 10 GPS dishes and 10 Nunc 60 mm dishes. Fifty microlitres of medium was underlaid into each of the eleven wells of each GPS dish, and eleven 50 μl microdrops of medium were underlaid into each of the Nunc dishes. The total preparation time was recorded for each set. All the dishes were cultured overnight at 37.3°C under 5% CO₂ in air.

On Day 1, 100 BALB/c X C57BL/6J zygotes were collected. The zygotes were randomly assigned to individual culture in either small GPS wells (5/dish, N = 50) or in microdrops (5/dish, N = 50). The time for the preparation of each dish was recorded.

On each of Days 2, 3, 4, and 5, the embryos were evaluated for development, and the time recorded for the examination of each dish.

The times required for set-up and embryo examination on each day were compared by Kruskal-Wallis tests. The proportions of zygotes that reached a given developmental stage on each day were compared by Chi-square analysis.
In standard microdrop culture, embryos are cultured in microdrops of medium on a flat surface.

**Results**
The time to set-up the cultures on Day 1 and the time required for embryo evaluation on Days 2, 3, 4, and 5 were all significantly less for GPS culture than for microdrop culture (Figure 3).

**Figure 3.** The times required for set-up and embryo examination in microdrops and GPS microwells.

![Bar chart showing time per dish (min) for microdrop and GPS cultures.](chart)

As shown in Figure 4, significantly larger proportions of embryos reached the morula stage and compacted on Day 3 in GPS culture than in microdrop culture. There were no differences in development on Days 2, 4, or 5.

**Figure 4.** Mouse embryo development in microdrops and GPS microwells.

![Bar chart showing % of zygotes for GPS and microdrop cultures.](chart)

**Conclusions**
The results show that mouse embryo development in GPS culture is as good as, or better than, development in microdrop culture, and that the times required for set-up of the cultures and embryo evaluation are significantly reduced in GPS culture compared with microdrop culture. The use of GPS dishes for human ART could significantly reduce labour time and costs while increasing security, especially for PGD embryo culture.
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A California company has brought human cloning research to a new level with efficient production of cloned human blastocysts – an early stage of embryos.

The company, Stemagen in La Jolla in California, hopes that its achievement will be the first step towards using cloning techniques for biomedical research and, potentially, therapy. But first they will need to go the next step – using such blastocysts to establish self-propagating lines of embryonic stem cells that, as clones, would be genetically identical to a patient.

Cloned human blastocysts have been reported before, but not at this level of achievement. The five cloned blastocysts produced by Stemagen are the first ones to be made with adult human cells – in this case male fibroblasts.

Korean researcher Woo Suk Hwang claimed in 2004 and 2005 to not only have created cloned human blastocysts, but also to have produced stem-cell lines from them. His results turned out to be fraudulent. In May 2005, Miodrag Stojkovic and his group at Newcastle University, UK, reported that three cloned embryos had made it to the blastocyst stage, but they could not produce a cell line. Stojkovic’s group used embryonic stem cells from discarded embryos from in vitro fertilization procedures, a less impressive achievement because these cells were already in a flexible embryonic state, and because they were not matched to any patient’s genetics.

Real, healthy clones?

Stojkovic, now at the Cellular Reprogramming Laboratory at Prince Felipe Research Centre in Valencia, Spain, and an associate editor at Stem Cells, says that this is a “huge difference” from what his group had achieved. Stojkovic also congratulated the group for doing extensive genetic tests. One of the blastocysts successfully went through rigorous tests to prove its identity. “After Hwang, the field is very sensitive,” says Stojkovic. “With these analyses, there is no doubt that at least one is a real clone.”

Robert Lanza from Advanced Cell Technology in Los Angeles, California, a competitor in the field, says that the article lacks data to show that the cells were fully reprogrammed and that the resultant blastocysts were in good condition. He says the photos of the blastocysts “look very unhealthy”.

Though most researchers have agreed not to use cloning techniques to produce human babies, there are fears that this sort of work might open the door to reproductive cloning says Marcy Darnovsky of the Center for Genetics and Society, a science watchdog group based in Oakland, California.

Good eggs

Lead author Andrew French credits the group’s success to the quality of the eggs they used. The company set up a laboratory next to a fertility centre and were able to get to work on eggs donated by women within two hours of extraction. Their 5 successful blastocysts came from 20-30 eggs.

Technologically there was nothing much new here, French admits. The team didn’t use the cutting-edge visualization technique that a group in Oregon claimed to be crucial for their success in creating an embryonic stem cell line from cloned monkey blastocysts, reported in November 2007 (see Cloned monkey stem cells produced [http://www.nature.com/uidfinder/10.1038/news.2007.24]).
Researchers are eager to see whether patient-matched stem-cell lines can be produced.

French says that there are no patents attached to their achievement. The company hopes to make money through agreements with drug companies that want access to specific stem-cell lines for different diseases.

Surprisingly, the group sent all five blastocysts out for independent DNA tests, foregoing a chance for the ultimate goal - the establishment of a cloned embryonic stem-cell line. French says they wanted to “make sure the right DNA was in the blastocyst” and rule out contamination. Since only 10-20% of such blastocysts are expected to produce cell lines, French says that he does not think they stood much of a chance anyway. “We would have loved to go for the holy grail and get the stem-cell line, but we wanted to get this first step sorted out first,” he says.

Harvard stem-cell expert George Daley describes the article as an “important first step” but he says the true test will be the derivation of cloned embryonic stem-cell lines.

Researchers in the field continue to take two parallel approaches to making patient-matched stem-cell lines. Some, like Stemagen, are using cloning; others are attempting to bypass eggs and embryos completely by instead reprogramming adult cells directly into embryonic-like stem cells.

References

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Glycyl-Glutamine
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The chemical instability of glutamine when dissolved in water has been known for many years. It decomposes into ammonium and pyrrolidine carboxylic acid particularly at the pH and ionic strength which exist in physiological solutions. The decomposition is also enhanced by heating. This instability creates a problem where glutamine is a component of physiological solutions used in both research and clinical medicine, because of the toxicity of ammonium to cells. If glutamine is coupled to another amino acid, such as alanine or glycine, by a peptide linkage a dipeptide is produced which is stable in aqueous solution and the solution can be heat-sterilized (Stehle et al., 1982).

The exploitation of the stability of glutamine-containing dipeptides as substitutes for glutamine in a physiological solution was first described in 1984. L-alanyl-L-glutamine was used to replace glutamine in a solution for parenteral administration in rats (Albers et al., 1984). Over the next few years several papers showing the effectiveness of these solutions was described. For example, Roth et al. (1988a) demonstrated that muscle loss of alanine and glutamine in post-operative anaesthetized dogs was reduced by the intravenous infusion of a physiological solution containing alanyl-glutamine. They state “In contrast to glutamine, the dipeptide alanylglutamine is stable in aqueous solution and therefore would be a suitable substrate for parenteral nutrition”. Karner et al. (1988) also described the parenteral administration of glutamine for the treatment of the sepsis state. They stated “We used glutamine dipeptides as sources because they remain stable in an aqueous state.” The use of stable glutamine-containing dipeptides to replace unstable glutamine is now used in human clinical medicine (Albers et al., 1988; review: Fürst et al., 1997).

An obvious extension from this work on the use of glutamine-containing dipeptides in physiological solutions for parenteral administration is to use these compounds in the preparation of media for the culture of cells in vitro. In fact, the use of alanylglutamine and glycylglutamine in media for the culture of a human cell line was described simultaneously by members of the same team who had worked on the design of the solutions for parenteral administration (Roth et al., 1988b). They wrote: “Both Ala-Gln (alanylglutamine) and Gly-Gln (glycylglutamine) have an advantage over free Gln (glutamine) as growth factors for cell culture due to the stability of the dipeptides during both autoclaving and storage; the biological activity, however, is comparable.” This work is consistent with the earlier studies by Eagle (1955) who showed that human cells in culture can utilize dipeptides. There are now several reports on the use of glutamine-containing peptides in media for the culture of cells (review: Biggers et al., 2004).

During the late 1990s alanylglutamine and glycylglutamine became available from Gibco Life Technologies under the brand names Gluta-
Definitions

Perenteral. Involving the introduction of a substance into the body other than by the alimentary canal.

Physiological solution. A mixture of chemicals dissolved in water for parenteral therapy or for the support of cells, organs or embryos in vitro.

Max-1 and GlutaMax-2, respectively. Among their marketing targets were investigators using “Culture systems especially sensitive to ammonia” (from advertisement).

The first report of the use of a glutamine-cotaining dipeptide in a medium for the culture of a preimplantation embryo was published by Hagemann et al., (1998), who cultured bovine embryos in a medium containing glycylglutamine (Glutamax-2). They found no differences between the growth of the embryos in media containing either free-glutamine or glycylglutamine. Their use of glycylglutamine was clearly to minimize the breakdown of glutamine in ammonium and pyrrolidone carboxylic acid, as shown by the following quote: “Because ammonia accumulation would also result from glutamine degradation, this could be avoided by the use of glycyl-glutamine (GlutaMax-2)…” Lane et al., (2001) replaced glutamine with alanylglutamine (Sigma) in a medium for the culture of mouse preimplantation embryos in order to reduce the production of ammonium. They wrote: “The increased stability of alanylglutamine results in the production of significantly less ammonium when incubated at 37°C”.

To summarize, the replacement of glutamine by a dipeptide containing glutamine has been used in three types of physiological solution: (1) in solutions injected into patients when this type of feeding is required (parenteral therapy), (2) in solutions (media) used to culture cell lines, and (3) in solutions (media) used to culture mammalian preimplantation embryos from the newly fertilized ovum (a single cell) to an organized aggregate of cells called the blastocyst.

The unique idea, common to all three applications, is to replace glutamine with a dipeptide containing glutamine to stabilize a physiological solution. Applying the strategy in a situation that requires a stable solution has nothing to do with the particular application involved. Once its usefulness has been demonstrated in one solution its application to another solution is intuitively obvious.

References


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The carousel of change is a fundamental tenet of life. Just as seasons turn on a yearly cycle and organisms evolve on a millennial timeline, our bodies have the amazing capacity to transform through regeneration. Over the ebb and flow of our entire lifetime, worn out cells die and are replenished by new cells so that we can continue to breathe, move, eat, smell, feel, read and think. We only need to consider our growing children, attend our 25th high school reunions, or look at earlier pictures of ourselves to realize that all of us inevitably, imperceptibly and imperfectly morph throughout our lives. But how and why does this happen? The answers may be found in our stem cells.

For most of us, the process of transformation is so gradual that we don’t seem to notice the progressive physical changes that occur on a daily basis. As you read this article, you will produce billions of new blood cells and millions of new skin cells. In fact, we continuously produce new cells for every organ and tissue; even the neurons of our brains are slowly replaced over time. Sunburned skin peels and is restored; broken bones mend; we can even sculpt our muscles much like the Play-Doh™ figurines we molded as children. With this plasticity comes the opportunity to direct cellular fates and dictate the biological outcome as we age. The basics of a healthy diet, sanitary habits and exercise can increase fitness, prolong life expectancy, and resist aging. With growing knowledge of the intricate mechanisms of developmental and regenerative processes, it may even be possible to create new medical treatments that either replace lost or damaged tissues or control fundamental aging processes. Imagine one day being able to take a pill that will enhance our body’s ability to regenerate tissue or inject cells that will rejuvenate or replace failing organs. With the help of modern science, will we find Ponce de Leon’s proverbial “Fountain of Youth” and prolong life expectancy?

In essence, regeneration is healing. We realize the healing power of nature, *vis medicatrix naturae*, as our inborn regenerative processes kick into high gear in response to injury. We scrape a knee and are appreciative of how quickly the body can heal itself. In a few short days, evidence of damaged tissue has vanished and the scraped knee is virtually forgotten. Underlying this outcome is a well-orchestrated sequence of events that begins with inflammation resulting in the removal of damaged tissue, followed by the generation of replacement tissue. Replacement cells, each with a specialized function for that tissue or organ, arise from a restricted reservoir of regenerative stem cells that exist in a specified niche in each tissue. A complex set of cues signal these stem cells to expand and then differentiate into the replacement cells needed to restore the damaged tissue. Thus, it is our stem cells that drive the regeneration process that is evident in healing. And it is our stem cells that reveal a diminishing ability to regenerate that ultimately results in aging.

Since stem cells are the facilitators of regeneration, understanding their origins, development and fates holds the key to discovering new mecha-
Since stem cells are the facilitators of regeneration, understanding their origins, development and fates holds the key to discovering new mechanisms and pathways that could result in the development of new regenerative medicines.

nisms and pathways that could result in the development of new regenerative medicines. Every cell of the trillions of cells that constitute our human bodies, including stem cells, can be traced back to the fertilized egg that came into existence with the initial union of sperm and egg. All of the over 200 different types of cells of our bodies are derived from a pool of stem cells in the early embryo. During embryonic development, as well as later in life, lineage-restricted stem cells give rise to the specialized or differentiated cells that perform specific functions of the body, such as skin, blood, muscle, and nerve cells. Not surprisingly, the same molecular instructions that guided our early development in utero also provide the biologic indicators that prompt differentiation decisions as we mature from childhood through adolescence into adulthood, as well as mediate the processes of homeostatic tissue regeneration and wound healing. Since the same biologic blueprint that specifies cell fate during early development is used again and again throughout our lives, a greater appreciation of developmental biology will provide important insights into the biology of stem cells.

Stem cells are found in all of us, from the early stages of human development to the end of life. All stem cells may prove useful for medical research, but each of the different types has both promise and limitations. Embryonic stem cells (ES cells), which can be derived from a very early stage in human development, are pluripotent, having the potential to produce all of the body’s cell types. Human ES cells have been the topic of much ethical debate in that current methods used for their isolation result in the destruction of pre-implantation human embryos. On the other hand, adult stem cells, which have been found in almost all tissues in fully developed humans, from babies to adults, may be lineage-restricted, limited to producing only certain types of specialized cells. Multipotent stem cells found in umbilical cord blood and matrix (Wharton’s Jelly), as well as the placenta and amniotic fluid, appear to be less restricted, giving rise to a wide variety of cell types and tissues. Recently, artificially-derived ES cell-like cells, termed induced pluripotent stem cells (iPS cells), have been generated by introducing key stem cell-associated genes into non-pluripotent, somatic cells such as skin cells or fibroblasts. These induction genes are thought to epigenetically re-program or de-differentiate somatic cells resulting in a pluripotent stem cell that is remarkably similar to an ES cell. While new delivery techniques and induction controls are still needed to increase the efficiency of the reprogramming method, iPS cells, created to have authentic ES cell-like properties without the controversial use of embryos, represent an important advancement allowing the derivation of patient-specific cells for therapeutic uses and disease-disposed cells for research applications.

The daily work that occurs in laboratories around the world studying stem cells begins with developing ways to identify stem cells, isolate cell lines in culture, and stimulate stem cells to differentiate. Self-renewing human ES cells are isolated from the inner cell mass of a pre-implantation blastocyst by placing these cells in a culture dish with nutrient-rich media that induces their replication in an undifferentiated state for very long periods of time. The largest source of blastocysts is from in vitro fertilization (IVF) clinics, but blastocysts can also be generated from eggs after nuclear transfer – the insertion of a nucleus from an adult somatic cell into a donated egg. Similar means are used to culture iPS cells after the re-programming step. Just a few human ES cells or iPS cells can build a large bank of stem cells to be used in experiments aimed to define the conditions needed for stimulating them to create specialized cells such as neurons, hepatocytes or cardiomyocytes. ES cells and iPS cells seem to be far more flexible than stem cells found in adults, because they have the potential to produce every cell type in the human body. ES cells are generally easier to collect, purify and maintain in the laboratory than adult stem cells. Improved derivation techniques may also make iPS cells easier to manipulate in the lab.

Adult stem cells are hidden deep in defined niches within organs, in times of need replenishing the millions of ordinary cells that surround them in that tissue. Some adult stem cells are currently being used in therapies such as bone marrow transplants or skin grafts. Though every organ has a characteristic turnover rate ranging from rapid (such as intestine, skin and blood) to slow (such as brain and pancreas), stem cells have been identified and isolated from every organ and tissue of the human body. Unlike ES cells, adult stem cells are
already somewhat specialized. Though adult stem cells may be lineage-committed, scientists are working on finding ways to stimulate adult stem cells to be more versatile. In addition, extra-embryonic material such as cord blood, matrix, placenta and amniotic fluid are being explored to provide alternative sources of multipotent, unspecialized cells.

As early as 1961, adult bone marrow was found to contain cells that could make all of the blood cell types. But it wasn’t until 1988 that these stem cells were isolated as pure populations. Why did it take so long? The techniques for identifying stem cells have only recently been developed. Partly, this is because adult stem cells are, by their very nature, inconspicuous in shape, size and function. They also tend to hide deep in tissues, don’t replicate unless needed and are present only in low numbers, making their identification and isolation like finding a needle in a haystack.

How do scientists know when they have found a stem cell? Every cell displays an array of proteins on its surface; different cell types have different proteins. Scientists can use the surface proteins as “markers” that characterize individual cell types—a type of “molecular fingerprint”. For example, using antibodies that recognize and attach to specific surface proteins that are chemically modified to fluoresce under specific wavelengths of light, scientists can visually tell the difference between a blood stem cell and a mature white blood cell. Unfortunately, not all stem cells can be currently identified in this manner because markers for all stem cell types have yet to be identified. Stem cells can also be identified by their behavior in the laboratory: stem cells must be able to remain unspecialized and self-renew for long periods of time. With new isolation techniques and improved methods to grow stem cells, the search for additional adult stem cell types continues.

Cell culture refers to the growth and maintenance of cells in a controlled environment outside an organism. Successful stem cell culture, keeping the cells healthy, dividing and unspecialized, is the first step in establishing a stem cell line—a propagating collection of genetically identical cells. Cell lines are important because they provide a long-term supply of multiplying cells that can be distributed for research and therapy development. However, over time, all cell lines change, typically accumulating harmful genetic mutations. Though there is no reason to expect that stem cell lines will behave differently, it is possible that the intrinsic nature of stem cells may enable them to maintain their genomic integrity via physiologic mechanisms that either resist or correct genetic mutations. While there is much that can be learned using existing stem cell lines, such concerns necessitate continued monitoring of these cells as well as the development of new stem cell lines in the future.

Once a stable stem cell line has been established, the process of causing the stem cells to differentiate into specialized cell types can begin. The cellular environment in which stem cells naturally reside provides clues as to how to make them differentiate in a culture dish. For example, in the bone marrow, where blood stem cells reside, bone cells send physical and chemical signals that tell the blood stem cells when to differentiate. The nature of these signals is just beginning to be understood so that ways to mimic the natural process in cell culture can be developed. Usually, the technology involves adding certain proteins to the cell culture and, in some cases, introducing specific genes into the stem cells. Significant advancements in this area have made it possible to generate a wide variety of stem cell-derived cell types such as neurons, hepatocytes and cardiomyocytes. These physiologically-relevant human cells have characteristics of their counterparts found in the human body and have demonstrated important functional properties making them a novel renewable source of human cells and tissues for transplantation, research and drug discovery.

Currently, only a few diseases are treatable with stem cell therapies because only a limited number of cell types can be efficaciously and safely regenerated. However, the success of the most established stem cell-based therapies—blood and skin transplants—gives hope that someday stem cells will allow physicians to provide therapies for a variety of diseases previously thought incurable. Many major diseases are caused by the loss of a single cell type or tissue. For example, type I diabetes (Juvenile-onset) is caused by the loss of the insulin-producing cells of the pancreas, and its treatment is limited to merely alleviating symptoms by giving the patient insulin replacement therapy. Finding a cure for such a disease would be much easier if it were possible to simply re-grow the missing or damaged cells and implant them into patients.

The list of medical achievements stem cells could offer seems to be expanding at an incredible pace. The role of stem cells in medicine is already very real, but there is a danger of exaggerating the promise of new medical developments. As scientists and physicians get caught up in the excitement of breakthrough discoveries, not only the potential outcomes of both embryonic and adult stem cell research are “over-promised”, but also the time scales that are involved. The basic research
needed to develop viable therapeutic options is a lengthy process that may extend over many years and decades. Even after science has moved from basic research to developing medical applications, it still takes many years to thoroughly test those applications and demonstrate that they are safe to prescribe for patients. This is true for all medical treatments, including the development of new drugs, procedures, and medical equipment, and is not specific to the living cell therapies made possible by stem cell research.

Stem cells offer opportunities for scientific advances that go far beyond cell-based regenerative medicine. They offer a window for addressing many of biology’s most fundamental questions. Watching embryonic stem cells give rise to specialized cells is like peeking into the earliest development of the many tissues and organs of the human body. Stem cell research may help clarify the role genes play in human development and how genetic mutations affect normal processes. They can be used to study how infectious agents invade and attack human cells, to investigate the genetic and environmental factors that are involved in cancer and other diseases, and to decipher what happens during aging.

Stem cells may also revolutionize traditional chemical medicine. Because stem cells can continue to divide for long periods of time and produce a variety of cell types, they could provide a valuable source of human cells for testing drugs or measuring the effects of toxins on normal tissues without risking the health of a single human volunteer. In the future, millions of compounds could be quickly tested on a wide assortment of cell types derived from stem cells, making drug discovery more efficient and cost effective. Access to a renewable source of human, physiologically-relevant, functional cells could revolutionize cell-based screening in all aspects of discovery and pre-clinical development of potential drugs.

Generating iPS cells from genetically-predisposed individuals could be particularly useful for testing drugs for disorders that are of genetic origin. For example, it is difficult to study the progression of Alzheimer’s and Parkinson’s diseases in the brains of live patients – but by using the cells from such patients to create iPS cell lines, it may be possible to trace the development of the disease in a culture dish and test drugs that regenerate lost nerve cells with no danger to the patient.

Since stem cells can serve as a model to study developmental or regenerative processes, they may also help scientists to calculate the effect of toxic substances in drugs, foods, and the environment. For example, a simple test measuring the differentiation of ES cells into beating heart cells is being used to identify potential teratogens that may cause reproductive toxicities. In addition, a slew of novel compounds and target proteins have recently been identified using stem cell models that modulate the regeneration of brain, bone, blood, fat, muscle, and heart tissues, providing the fodder for developing prospective new regenerative drugs.

Currently, all forms of stem cell research in the U.S. are legal at the federal level. That is, it is not illegal to make or work with new embryonic stem cell lines. However, the use of federal funds for human embryonic stem cell research is restricted to the “Presidential” cell lines that were available before August 9, 2001. Therefore, the derivation of new embryonic stem cell lines can only occur when scientists are working with non-federal funding. Many prominent scientists and physicians have publicly taken the stand that stem cell technology is too important to be limited by restrictions on any lines of investigation. To take up the slack, some states and private foundations have been supporting the work that is currently encumbered by federal funding limitations. Appropriately, some requirements of federal law, such as protection of human subjects, apply to state- and privately-funded stem cell research. With changing political winds beginning to blow, perhaps it is time for us to realize that the true power to unleash the enormous potential of the stem cell lies within us, both morally and scientifically.
In the early days of mammalian embryo culture, it was believed that embryos in culture either died or else recovered to develop normally. Austin (1973) expressed this view in saying:

“The effect (of teratogenic agents) on the cleavage embryo depends on the number of cells killed or inhibited: above a certain proportion, the embryo dies; below that figure the remaining cells multiply to replace those lost and subsequent development is essentially normal.”

We now know that this view was wrong. Exposure to a variety of agents and environmental conditions during in-vitro culture can have significant effects on development after transfer to the uterus, and, most importantly, with few or no readily observable immediate effects. As for my previous articles for Fertility Magazine, this is not intended to be a comprehensive review of the topic, but rather a brief overview, using selected illustrative references. Moreover, much of the investigation of the long-term effects of in-vitro embryo culture has focused on attempting to identify the molecular mechanisms, including disturbances of genetic imprinting. The discussion here will be limited to observations of the developmental and physiological effects of a few of the many possible effectors. For comprehensive reviews of the topic, see Johnson (2005), Pool (2005) and Thompson (2007).

Perhaps the earliest report of effects of in-vitro exposure of embryos to an agent on subsequent development was the report of the effects of methylnitrosylurea (MNU) on mouse embryos by Iannaccone (1984). Mouse blastocysts were exposed to various doses of MNU for one hour, and the surviving embryos transferred to recipients. A dose of 4200 μg/kg MNU was required to kill 50% of the embryos immediately after exposure, but a dose of only 4.7μg/kg, 1000-fold less, reduced the live-birth rate by 50%. This clearly showed that a dose of MNU too low to have an immediate effect could have a significant later effect. Even more striking was the effect on post-natal survival. As shown in Figure 1, there was a significant perinatal loss of pups derived from MNU-exposed embryos compared to controls, and further losses until, at 52 weeks, the crude mortality rate was 58% for the MNU group compared with 22% for the controls. Interestingly, there were no chromosomal, histological, or anatomical abnormalities associated with the MNU treatment.

Willadsen et al. (1991) reported a high incidence of congenital deformities and very high birth weights among calves produced by cloning. This was later also shown to occur in calves and lambs produced by in-vitro maturation, fertilization and culture (IVP), and is known as the Large Offspring Syndrome. Figure 2 shows the results of a meta-analysis by Kruip and den Daas (1997) in which gestation length, birth weight, dystocia score and perinatal death rate were significantly greater in calves resulting from embryos produced by IVP compared with calves resulting from transfer of in-vivo-produced embryos. Clearly, although apparently benign, in-vitro procedures can have significant effects on subsequent development.

It is well recognized that atmospheric concentrations of oxygen (20%) can have detrimental effects on...
In the early days of mammalian embryo culture, it was believed that embryos in culture either died or else recovered to develop normally.

Figure 1. The effect of exposure of mouse blastocysts to methyltrinitrobenzene (MNU) on post-natal mortality. (Redrawn from Iannaccone, 1984)

Figure 2. Comparison of gestation length, birth weight, dystocia score and perinatal death rate in calves produced by artificial insemination (AI), transfer of in-vivo produced embryos (ET) or in-vitro maturation, insemination and culture (IVP). (Kruip and den Daas, 1997)

Figure 3. The effect of oxygen concentration on the viability of human embryos (Catt and Henman, 2000)

Figure 4. The effect of glucose concentration on the development to blastocyst and the sex ratio of cattle embryos (Kimura et al., 2005)

detrimental to the development of mammalian embryos in vitro. Notably, Thompson et al. (1992) showed that glucose concentrations greater than 3.0 mM inhibited the development of sheep embryos. Media for human embryo culture usually contain only low to moderate concentrations of glucose. Interestingly, however, it has been shown that glucose may be more detrimental to female embryos than to male embryos, such that exposure to even moderate concentrations of glucose in vitro may result in increased proportions of male embryos. For example, as shown in Figure 4, Kimura et al. (2005) found that although 2.5 mM glucose had no effect on the overall proportion of 8-cell cattle embryos that developed to the blastocyst stage, that concentration of glucose significantly increased the proportion of male embryos.

Cohen et al. (1997) found significant concentrations of a wide variety of volatile organic compounds (VOC)
in the atmosphere in their IVF unit in New Jersey. Surprisingly, the concentrations of many of these VOC were higher in the laboratory than in outside air, indicating that they arose within the laboratory or from adjacent areas. Possible sources included anesthetics, fumigants, adhesives, laboratory plastics and incubator gases. They were able to relate changes in air quality to implantation and clinical pregnancy rates, even though there were no significant effects on embryo development or morphology. Cohen and Dale subsequently designed small air filtration (Coda) units to be placed within the incubator. Mayer et al. (1999) found that the use of Coda units resulted in increased pregnancy rates, but without any apparent improvement in embryo quality. Using cattle embryos, Merton et al. (2007) similarly found that the use of Coda units significantly improved pregnancy rates, but had no effect on the rate of development to blastocyst or on embryo quality (Figure 5).

In conclusion, it is becoming increasingly clear that the in-vitro environment can have deleterious effects on short and long-term embryo development and even post-natal life. However, it is important to note that the effects of specific environmental conditions differ between studies, and not all embryos will be affected by any given effector. This suggests that the ultimate fate of the embryo depends on its inherent resistance to environmental stress and the sum of the environmental stresses to which it is exposed. Therefore all factors must be evaluated before introducing them into common ART practice. The use of maternal serum for in-vitro oocyte maturation (IVM) is a case in point. Thompson et al. (1995) found that the use of human serum in sheep embryo culture medium significantly increased the incidence of large lambs, and Calder et al. (2004) showed that serum significantly inhibited the expression of a number of genes in cattle cumulus-oocyte complexes. Because it is an undefined component, the use of maternal serum has largely been abandoned for human embryo culture, and the same concern should apply to its use for IVM.

Figure 5. The effect of Coda air filtration on the development of bovine embryos to the blastocyst stage and on pregnancies rates (Merton et al., 2007).

References
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HEPA Is Not Enough

Most embryologists are familiar with the HEPA (High Efficiency Particulate Air) filters used in laminar flow hoods and, in some cases, in central air supply systems (Figure 1). Interestingly, HEPA filters do not resemble a sieve, but rather are a mat of bound fiberglass fibers, with approximately 10-micron spaces between the fibers (Figure 2). Particles in the air stream adhere to, or become imbedded in, the fibers, and HEPA filters will trap almost all particles greater than 0.3 microns in diameter. This is sufficient to remove airborne particles, fungi, mold spores, and bacteria, and therefore HEPA filters can significantly reduce microbial contamination. However, HEPA filtration is not enough, and will never be enough, for the stringent air quality requirements of human ART.

Molecules of embryotoxic compounds are 100 to 1000 times smaller than the effective pore size of a HEPA filter. Volatile organic compounds (VOCs) such as benzene, formaldehyde, acetaldehyde, acetonitrile have been found in laboratory air (Hall et al. 1998), and they are not trapped by a HEPA filter. To trap or destroy these materials requires a smaller trap, such as is provided by activated charcoal. The spaces between the carbon particles contain a cloud of delocalized electrons that acts as an electronic glue (van der Walls forces), to bind chemical contaminants onto the carbon. Compounds such as alcohols and ketones are not easily removed by carbon, but they can be oxidized, and thereby detoxified, by potassium permanganate. Again, this is a chemical reaction happening at a scale of a thousand times smaller than the particles trapped by a HEPA filter. The essential point is having an effective removal device that fits the scale of the particle or chemical molecule.

The possible significance of air quality on IVF was raised by Cohen et al. (1997) who observed decreases in in-vitro embryo development and pregnancy rates associated with the move of an IVF lab from suburban Naples, Italy to the downtown area in 1992, and associated with construction around another IVF lab in New Jersey in 1995. Some environmental effects from other events involving construction in neighbouring spaces and the use of toxic materials in lab spaces preceded these periods. They consequently measured significant concentrations of VOCs in the laboratory air, and in the compressed CO₂ used for the incubators. Based on these observations, they designed the Coda® incubator units for use inside the incubators, in-line Coda® filters for the CO₂ supply, and larger Coda® towers to filter the laboratory air, all of which con-
HEPA filtration is not enough, and will never be enough, for the stringent air quality requirements of human ART.

tain HEPA filters, activated charcoal and potassium permanganate.

A number of studies have shown improved pregnancy rates with the use of Coda® air filtration in human IVF labs (Racowsky et al. 1999, Figure 3; Mayer et al. 1999, Figure 4) and cattle IVF (Merton et al. 2007, Figure 5). It is important to note, however, that it is not realistic to expect the use of Coda units to improve in-vitro development and/or pregnancy rates in every case. A difference in clinical outcome is only likely if there is an ongoing problem with air quality, or if there is a dramatic decrease in air quality, from nearby construction, for example, during the study period. Such environmental crises can and do occur, but they are often intermittent and rarely predictable.

In conclusion, HEPA air filtration is highly effective for the reduction of particulates and bacteria, but cannot reduce the concentrations of embryotoxic VOCs in the ART laboratory. Coda® filters contain activated charcoal and potassium permanganate and can significantly reduce VOCs. Coda® units should be considered as common-sense safety devices. That is, they should always be in place, in order to deal with unforeseeable changes in air quality.

References
HARTFORD -- The State of Connecticut Stem Cell Research Advisory Committee yesterday directed the allocation of $9.84 million in stem cell research funds to investigators based in Storrs, Farmington and New Haven.

This is the second installment of grants from the Stem Cell Research Fund, established by the Connecticut General Assembly and signed into law by Governor M. Jodi Rell in 2005. Between now and 2015, the committee is tasked with allocating approximately $100 million in order to encourage stem cell research in Connecticut.

“While other states are cutting back on their financial commitments to stem cell research, Connecticut remains at the forefront of investing in and supporting cutting edge research in this emerging field,” said the Governor. “Connecticut once again proves itself as a nurturing and supportive environment where a variety of stem cell research can move forward safely, ethically and effectively, with an eye toward investing in the overall improvement of the public health of future generations.”

Eighty-seven stem cell funding applications were accepted for consideration in November 2007. From December through February, a 14-member Connecticut Stem Cell Peer Review Committee reviewed these applications in accordance with National Institutes of Health guidelines and provided to the Advisory Committee its recommendations with respect to the scientific merits of each application.

“It has always been the intent of the Connecticut Stem Cell Research Advisory Committee to fund the best stem cell research proposals that Connecticut scientists can offer,” said Stem Cell Research Advisory Committee Chairman and Department of Public Health Commissioner J. Robert Galvin, M. D., M. P. H., M. B. A. “After careful consideration and review by both an international panel of experts and by this Advisory Committee, we are confident that we have been true to our charge, and that these investments will yield significant scientific findings in the long-term.”

In its first round of funding in November 2006, the committee directed the allocation $19.78 million to 21 stem cell projects at Yale, UCONN and Wesleyan University.

“The stem cell research that this year’s grant recipients will undertake is meaningful to the state not only because of its potential public health benefits but also because it may lead to significant economic development in Connecticut,” said Peter Longo, president and executive director of Connecticut Innovations, which assists with administration of the Stem Cell Research Fund. “It is our hope that the scientific findings of the research will be refined for market introduction by existing and new Connecticut bioscience companies, helping further expand our growing bioscience sector.

These stem cell grant allocations are expected to fund the following projects:

Maintaining and Enhancing the Human Embryonic Stem Cell Core at the Yale Stem Cell Center, Yale University Stem Cell Center, New Haven, Haifan Lin, PhD, Principal Investigator, $1,800,000.00.

FOR IMMEDIATE RELEASE
Tuesday, April 2, 2008
Connecticut Department of Public Health
Contact: William Gerrish
(860) 509-7270
State of Connecticut prepares to allocate $9.84 million in stem cell research funds

Traslational Studies in Monkeys of hESCs for Treatment of Parkinson’s Disease, Yale University School of Medicine, New Haven, D. Eugene Redmond, Jr., MD, Principal Investigator, $1,120,000.00.

Establishing the Connecticut Therapeutic Cloning Core Facility – From Startup Technology/Feasibility Tests to SCNT/hESC Derivation Services, Evergen Biotechnologies, Inc., Storrs, Jang-Won Lee, Principal Investigator, $900,000.00.

Production and Validation of Patent-Matched Pluipotent Cells for Improved Cutaneous Repair, University of Connecticut Center of Regenerative Biology, Storrs, Theodore Rasmussen, PhD., Principal Investigator, $634,880.00.

Tyrosone Phosphorylation Profiles Associated with Self-Renewal and Differentiation of hESC, University of Connecticut Health Center, Farmington, Bruce Mayer, PhD., Principal Investigator, $450,000.00.

Directed Differentiation of ESCs into Cochlear Precursors for Transplantation as Treatment of Deafness, University of Connecticut Health Center, Farmington, D. Kent Morest, MD, Principal Investigator, $450,000.00.

Targeting Lineage Committed Stem Cells to Damaged Intestinal Mucosa, University of Connecticut Health Center, Farmington, Daniel W. Rosenberg, PhD., Principal Investigator, $450,000.00.

Modeling Motor Neuron Degeneration in Spinal Muscular Atrophy Using hESCs, University of Connecticut Health Center, Farmington, Xuejun Li, PhD., Principal Investigator, $450,000.00.

Human Embryonic and Adult Stem Cell for Vascular Regeneration, Yale University School of Medicine, New Haven, Laura E. Niklason, MD, PhD, $450,000.00.

Effect of Hypoxia on Neural Stem Cells and the Function in CAN Repair, Yale University, New Haven, Flora M. Vaccarino, Principal Investigator, $449,771.40.

Wnt Signaling and Cardiomyocyte Differentiation from hESCs, Yale University, New Haven, Dianqing Wu, Principal Investigator, $446,818.50.

Flow Cytometry Core for the Study of hESC, University of Connecticut Health Center, Farmington, Hector Leonardo Aguila, PhD., Principal Investigator, $250,000.00.

Cortical neuronal protection in spinal cord injury following transplantation of dissociated neurospheres derived from human embryonic stem cells, Yale University School of Medicine, New Haven, Masanori Sasaki, MD, PhD, Principal Investigator, $200,000.

Molecular Control of Pluripotency in Human Embryonic Stem Cell, Yale Stem Cell Center, New Haven, Natalia Ivanova, Principal Investigator, $200,000.00.

Cytokine-induced Production of Transplantable Hematopoietic Stem Cells from Human ES Cells, University of Connecticut Health Center, Farmington, Laijun Lai, PhD, Principal Investigator, $200,000.00.

VRK-1-mediated Regulation of p53 in the Human ES Cell Cycle, Yale University, New Haven, Valerie Reinke, Principal Investigator, $200,000.00.

Definitive Hematopoietic Differentiation of hESCs under Feeder-Free and Serum-Free Conditions, Yale University, Caihong Qiu, PhD, Principal Investigator, $200,000.00.

Differentiation of hESC Lines to Neural Crest Derived Trabecular Meshwork Like Cells – Implications in Glaucoma, University of Connecticut Health Center, Farmington, Dharaminder Choudhary, PhD., Principal Investigator, $200,000.00.

The Role of the piRNA Pathway in Epigenetic Regulation of hESCs, Yale University, New Haven, Qiaoqiao Wang, PhD., Principal Investigator, $200,000.00.

Early Differentiation Markers in hESCs: Identification and Characterization of Candidates, University of Connecticut Center for Regenerative Biology, Storrs, Mark G. Carter, PhD., Principal Investigator, $200,000.00.

Regulation hESC-derived Neural Stem Cells by Notch Signaling, Yale University, New Haven, Joshua Breunig, MD, Principal Investigator, $188,676.00.
CONFERENCES

The Sheraton Keauhou Bay Resort and Spa
First World Congress on Reproductive Biology
May 24-26, 2008
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27-30 May 2008
Sheraton Keauhou Bay Resort and Spa
Kailua-Kona, Hawaii
CONFERENCES

7th ISSCR Annual Meeting
International Society for Stem Cell Research

July 8-11, 2009
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