POTENTIAL APPLICATION OF NANOTECHNOLOGY IN EPIGENETICS

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PASS WITH MERIT
(830 words over limit)

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Abstract
This paper investigates nanotechnology as a solution to allowing bi-parental parthenogenesis to occur in humans. As the genetic application of nanotechnology is an already dynamic area of study with much promise for the future, I was attracted to the idea of investigating its application in this field further, in order to allow those who would otherwise be unable to create progeny to do so. I concluded that use of an AFM with a nano-manipulator add-on was the best method of demethylation in order to reverse the effect of genomic imprinting, but discovered that the interference in the creation of offspring, be it for the good of the embryo or to satisfy intellectual curiosity, would make it a controversial topic which could make such reproductive technology difficult to introduce.

Introduction

At the mention of the word 'nanotechnology', buckminsterfullerene's and nano-machines spring to the mind immediately, but assuming that nanotechnology consists of such technologies exclusively would be greatly underestimating the variety of the subject; nanotechnology is an incredibly wide science concerning any technology at the nano-scale (1nm=10^{-9}m), offering a great range of applications in medicine and also much potential for development by innovative scientists in the future. It is only in the past 30 years that we have had the knowledge and technology available to be able to investigate the behaviour of substances not only at the cellular level, but at the molecular and atomic level.

The precedence of fullerenes in many developments in nanotechnology may cause other methods of drug delivery and medical imaging on the nano-scale to be overlooked. For example, a much simpler method of specific drug delivery would be the use of amphiphiles to create vesicles with a similar structure to natural phospholipid-bilayer bound vesicles; the hydrophilic head of the molecule facing outwards, whilst the hydrophobic tails face inwards (Fig-1). The ability of amphiphiles to move freely within the plane of the membrane allows drugs to be injected into the vesicle to form liposomes, the contents of which can then be absorbed into cells by adhesion and then diffusion or endocytosis. The amphiphiles involved need not be phospholipids; polymers may instead be used to increase durability, stability, flexibility and to decrease the risk of accidental release. This could also allow trigger molecules to be attached to the vesicle wall, enabling release to occur in response to a specific cell. On one hand, liposomes have already proved useful in drug delivery of doxorubicin, allowing treatment of ovarian cancer and Kaposi's sarcoma, as well as insulin release in diabetics. On the other hand, liposomes trigger an immune response which leads to their destruction. However, it has been discovered that presence of the synthetic polymer poly-(ethylene glycol) in the liposome reduces phagocytosis; they become 'stealth liposomes'.

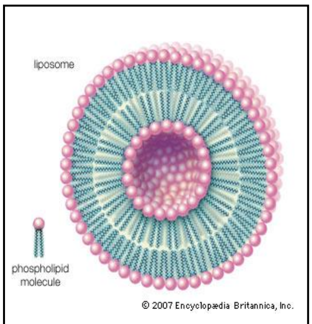


Figure 1

Focusing on applications in genetics, one way in which nanotechnology had already played a part in this area is in allowing scientists to identify genetic sequences, by, for example, the use of DNA micro-arrays. The methodology involves isolation of mRNA from two cell samples (for example, one diseased and one not), followed by use of complementary base pairing to create cDNA and then adding the sample to the micro-array (small, solid chips on which DNA, cDNA and oligonucleotides may be placed) after which hybridisation takes place . Following hybridisation, the micro-array may then be placed in a scanner consisting of lasers, a microscope and camera, allowing an analysis regarding which genes are activated and which repressed to be carried out by simultaneous comparison of the two samples. This enables the researcher to compare gene expression between control tissue and diseased tissue, and thus plays a vital part in pharmacogenomics, which may lead to the possibility of tailor-made drugs based on the genetic make up of the individual. More recently, Oxford Nano-pore technologies have developed 'exonuclease' and 'strand' DNA sequencing, allowing bases to be identified by their current as they pass through a 'nano-pore' (a small hole made out of protein).

Another exciting area of nanotechnology is the microscopy which allows us to see on the nano-scale, for example the AFM, which involves a small Si or Si_3N_4 probe on a micro-machined cantilever, mapping the contours of a material as it moves up and down. A laser is deflected (resultant of the force between the tip and the sample, which can be calculated by use of Hooke's law) off the surface of the AFM lever, which is then detected by a position sensitive photo-detector (Fig-2). What makes the AFM stand out above other microscopes such as the TEM and SEM is not only its ability to function outside a vacuum, but its ability to manipulate atoms, allowing modification of molecules. The nano-manipulator developed by 3rd Tech has even proved able to modify the structure of individual adenoviruses and DNA strands, employing the AFM to manifest the 3-D structure of the sample, then using computer graphics and haptics displays to allow the user to manipulate the actual sample in real-time on the nano-scale.

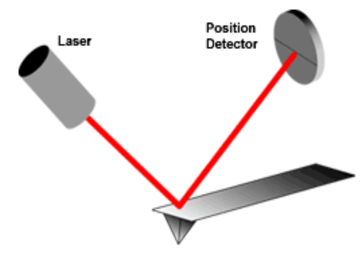


Figure 2

I have by no means covered the applications of nanotechnology in medicine; only those relevant to my discussion concerning epigenetics. Other areas of interest include functionalised buckminsterfullerene’s, nano-robotics, quantum dots and nano-fluidic ‘lab-on-a-chip’s.

Discussion

In this paper I shall be focusing on applications of nanotechnology in genetics, an area in which its use is already well established through its role in genomic mapping. It was emphasised by Lewis Wolpert in 'Triumph of the Embryo' (1991) that, “For development, the importance of explanations at the molecular level is that genes act at this level, […] it is at the level of molecular interactions that cells and embryos need to be understood. Molecules are the natural language of the cell.” Hence, for a true understanding of genetics and development, it is necessary to contemplate genes at the molecular level: the nano-scale. In particular, I shall be focusing on the possibility of enabling bi-parental parthenogenesis to occur.

Background Science and Proposal

Parthenogenesis, a process by which multi-cellular organisms may asexually reproduce, is observed in certain birds, reptiles and bony fishes and sharks, allowing offspring to be born without the acquisition of male gametes. Parthenogenetic reproduction generally occurs in favourable environmental conditions, where variation in the population is less likely to result in natural selection. The ova from which the 'clones' develop are often diploid, producing offspring genetically identical to the mother. Uni-parental parthenogenesis has been a phenomenon investigated for as long as a century; in 1900, Jacques Loeb reported that he had been able to induce growth of unfertilised frog eggs through scratching them with a needle, and then in 1914 by exposing unfertilised *Arbacia* and *Chaetopterus* to ultraviolet light in hypertonic water he stimulated development into larvae. In 1990, even unfertilised rabbit ova were prompted to segment, by delivering electric field pulses to the plasma membrane every four minutes in an isotonic glucose solution containing calcium ions. However, the resultant embryos would consistently develop abnormally and inevitably perish. In 2004 the problem was identified by a team of scientists working in Tokyo: in order for parthenogenesis to occur in mammals, bi-parental reproduction would be necessary due to the effect of genomic imprinting. By use of ova acquired from mutant mice with the appropriate expression of genes such as Igf2 and H19, viable mice were carried to full term, which themselves were able to reproduce normally once they reached maturity. Dr Kono, one of the scientists who developed the experiment, commented that, “It is impossible to do this experiment in a human”. I shall be investigating means by which bi-parental parthenogenesis could be carried out in humans, but through the use of nanotechnology.

Ideally, the amalgamation the two ova pro-nuclei would result in a diploid zygote, after which normal development would occur. However, this is not the case as a consequence of genomic imprinting, which affects the expression of genes depending on the parent from which it was inherited. The effect of genomic imprinting can be observed by the contrasting manifestation of the symptoms expressed by Prader-Willi and Angelman syndrome, both of which occur as a result of the deletion of a small segment of chromosome 15. Nevertheless, whereas Prader-Willi syndrome, in which the abnormal chromosome is inherited from the father, presents itself as hypotonia and insatiable hunger, Angelman syndrome, in which the abnormal chromosome is inherited from the mother, results in severely delayed development but a characteristic happy disposition. If it were not for genomic imprinting, the alleles from the other parent would instead be expressed, but as a result of imprinting the genes are 'silenced'.

In the 1980s, two groups of scientists based in Philadelphia and Cambridge allowed ova to be fertilised by spermatozoa, and then used a pipette to swap the pro-nuclei, resulting in two sets of viable zygotes; those from two ova and two spermatozoa. However, it was found that in both cases the embryos failed to develop properly; whilst those who had inherited maternal DNA formed an ordinary embryo proper but could not establish placenta, zygotes formed from two spermatozoa developed into a headless ball of cells surrounded by healthy extra-embryonic tissue, each hinting at the roles of paternally and maternally expressed genes. As a result it was suggested by David Haig that the imprinting of genes is a result of evolutionary conflict: paternally expressed genes produce a placenta invasive enough to ensure growth against the benefit of the mother, to whom it would be disadvantageous to invest all her resources in one child.

This was supported by the discovery of two genes expressed in foetal mice: Igf2, a paternally expressed gene, and Igf2r, a maternally expressed gene. When Igf2 is disabled the result is mice weighing 40% below average, suggesting a link with growth. However, when Igf2r is disabled the mice are born 125% heavier than average, suggesting a function to moderate growth. Although Igf2r is not present in humans, the gene H19 instead acts to moderate the growth resultant of Igf2 (which can increase the risk of adrenocortical carcinomas where H19 expression is low, or Beckwith-Weidemann syndrome where two paternal copies are accidentally inherited). If all genomically imprinted genes are of the same nature, theoretically elimination of all imprinting should result in a normal embryo; whilst, for example, they would have twice the number of Igf2 genes, they would also have twice the number of H19 genes. Ideally, traditional Mendelian genetics concerning one allele's dominance over another would also come into play if imprinting was eliminated.

Predominantly the method through which the genomically imprinted genes are silenced is by the addition of a methyl group to the 5-carbon position on cytosine, the transfer of which from S-adenosylmethionine is catalysed by methyltransferase (Fig-3). Other factors which may contribute to genomic imprinting include modification of histones, the timing of DNA replication and intra-nuclear location (chromosomal regions containing silent genes tend to occupy positions close to the nuclear envelope, whilst active genes are found more frequently in the nuclear centre). If one was to reverse all methylation, it would simply be necessary to competitively inhibit the methyl transferase enzyme with a substance of a similar shape to SAM-CH_3, which, after cell division, would result in passive demethylation. However, there are two obstacles that would make this practice counterproductive. Firstly, genomic imprints resist all passive demethylation; demonstrated when between fertilisation and implantation, DNMT1o (an ovum specific form of methyl-transferase) is excluded from the nucleus. The few sites of paternal imprinting are even able to resist active demethylation of the paternal genome in the zygote, and some placental genes possess methylated histones, allowing them to maintain imprinting without the action of methyltransferase. Secondly, methylation of cytosine also acts as a mechanism of suppressing transposons, "jumping genes" such as Alu and LINE-1 which are otherwise able to mutate other genes through their mobility, causing conditions such as haemophilia. Whilst only 0.14% of mutations in humans and 10% in mice are caused by "jumping genes", considering that there are over twice as many human endogenous retroviruses as coding-DNA, 45% of genes are derived from transposons and that 14.6% genome is made up LINE-1s, blindly demethylating the whole of the genome could present a significantly high risk.

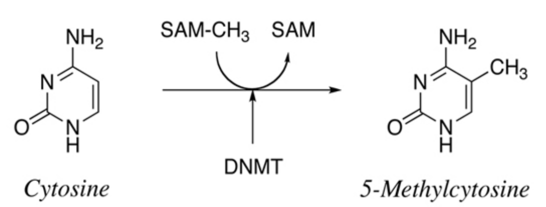


Figure 3

Hence, to reverse genomic imprinting great precision is required; precision that I propose can be attained through nanotechnology. One way in which genomic imprinting could be reversed would be by mapping of genes in the gametes of the participants, and then building up the nucleic acids from scratch through oligodeoxynucleotide synthesis (Fig-4). However, at the present time artificial synthesis of DNA is only capable of producing a short polymer made up of fewer than fifty nucleotides, compared to one chromosome being made up of up to 200 million base pairs. Furthermore, methylation of "jumping genes" would have to take place in order to reduce the risk of mutation, of which there are a greater number than that of the genomically imprinted genes.

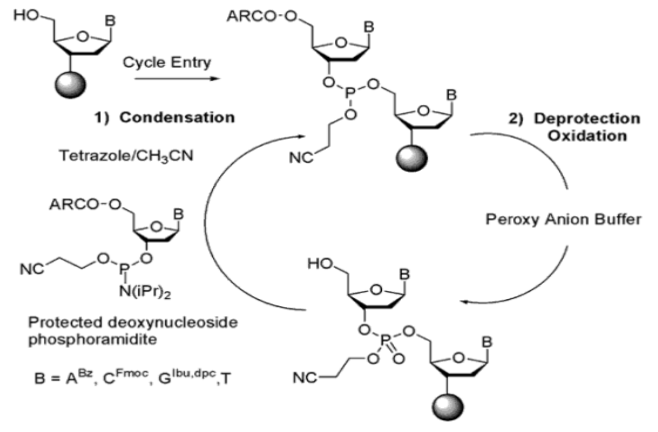


Figure 4

Thus, a more sensible approach would be the isolation and modification of already existing DNA, using techniques far more familiar in the realms of nanotechnology. The first step would be to identify the genomically imprinted areas by analysis of either gene expression using micro-arrays or methylation using bisulphite sequencing (which converts non-imprinted cytosine to thymine, leaving methylated cytosine unchanged). If, for example, two women wished to partake in bi-parental parthenogenesis, a comparison could be made between the expression of their gametic genes and those of a donated spermatozoon. If a gene is exclusively expressed in the ova, it would be paternally imprinted. If a gene is only expressed in the spermatozoon, it would be maternally imprinted and should be located on the ova DNA to undergo modification.

Once the exact whereabouts of the genomically imprinted genes are identified, I propose that the main step, where precise demethylation occurs, should revolve around the use of an Atomic Force Microscope. Once the DNA sequencing has identified the general area of the methylation, the sample could be placed in an Atomic Force Microscope in the appropriate positioning in order to confirm the presence of a methyl group. Using the force-feedback pen developed by 3rd Tech, Inc, or a similar nano-manipulator, it would be possible to remove the atoms one by one. The main limitations of nano-manipulators are their inability to precisely assemble atoms, and the difficulty of separating the atoms on the manipulator hand and the atom picked up from the sample. However, in this case these limitations present no problem; we are only interested in the removal of the methyl group, and the 'stickiness' of the manipulator hand is indicative that the force is strong enough to thoroughly separate the atoms from the genes. However, the AFM 'only' has a resolution of 1nm, whereas the covalent diameter of carbon is only 0.152nm and of hydrogen 0.062nm; hence, in order for the removal to take place, the manipulator hand would have to be positioned at an estimate of the whereabouts of the methyl group, using knowledge of the bisulphite sequencing results, covalent radii and electron-repulsion theory.

Once demethylation of the imprinted genes is complete, both sets of gametic genes should be reintroduced into their respective ova cells, either through micro-injection, electroporation (in which an electrical impulse temporarily creates a hole in the membrane and allows the DNA to pass through) and liposomes (as mentioned in the introduction). Alternatively, only the short stretches of imprinted DNA could be created by oligodeoxynucleotide synthesis, and then introduced to the nucleus in the same way, hoping that the behaviour of DNA replication will be unaffected. Subsequently, each of the ova would then be fertilised by a spermatozoon without allowing fusion of the pro-nuclei, then exchanged so that fusion of the two ova takes place to form a zygote which can then be implanted in the uterus of the woman.

The Ethics

The first IVF birth took place 33 years ago; the genome project was launched 23 years ago and the first mammal, Dolly, was cloned 15 years ago. Whilst reproductive technologies have greatly advanced, techniques such as IVF still evoke controversy, and it is likely that individual attitudes to new advances in reproductive technology would be formed on a similar basis.

There are a range of disadvantages which could be employed as an argument to reject the prospect of bi-parental parthenogenesis. Firstly, there are the religious reasons; contributing significant opposition; 71.6% of Britons and 78.5% of Americans follow the Christian faith. The central source of dispute would be that concerned with the unnatural nature of the procedure; the interference with genetics and embryos could certainly be interpreted as intruding in the creation accomplished by God and breaching the sanctity of life. Whilst IVF acts as a 'helping hand', allowing couples normally unable to conceive to do so, creation of an embryo from two parents of the same gender is a situation which would never naturally occur in mammals, and would hence be likely to generate a greater amount of controversy. A potential secondary source of opposition by fundamentalist Christians (and other conservative religious groups) may be the firm disapproval of homosexuality.

In *New Scientist* (February 12th 2011), Philip Ball bleakly commented that, "Anthropoeia [creation of people] will be used for social engineering by dictators – or indeed to duplicate themselves; it will lead to the demise of the family; it will result in the annihilation of men". He highlights the root of discomfort concerning reproductive technology; the notion that we can be 'manufactured', a fear fuelled by works of fiction such as *Frankenstein* (1818) and *Brave New World* (1932). Such novels suggest creation evolving into a cold, heartless procedure; "devoid of human value and subject to the dictates of science and technology" in the words of Pope John Paul II.

Secondly, there is the question concerning whether it is necessary to have two parents: one male, and one female, to be raised in an appropriate way, the answer of which has already been demonstrated by the rapid decrease in nuclear families: in May 2009 *The Independent* reported that according to the Office of National statistics, the percentage of households categorised as traditional nuclear families have dropped from 52% to 36%. It should be considered that although there are those who oppose the parenthood of same-sex couples, single parents are generally accepted as being competent (lone parents account for 9.6% of households in the UK, 90.5% of which are women), even though they only offer the influence of one gender. Acknowledging that 47.8% of women and 62.9% of men classed as lone parents are employed, the notion of same-sex couples as unable to provide rounded care for children should sensibly be discarded, as they would most likely between them be able to provide more time to care for their children. Since 2005, gay and lesbian couples have been able to adopt, reflecting changing attitudes and increased acceptance for non-typical families.

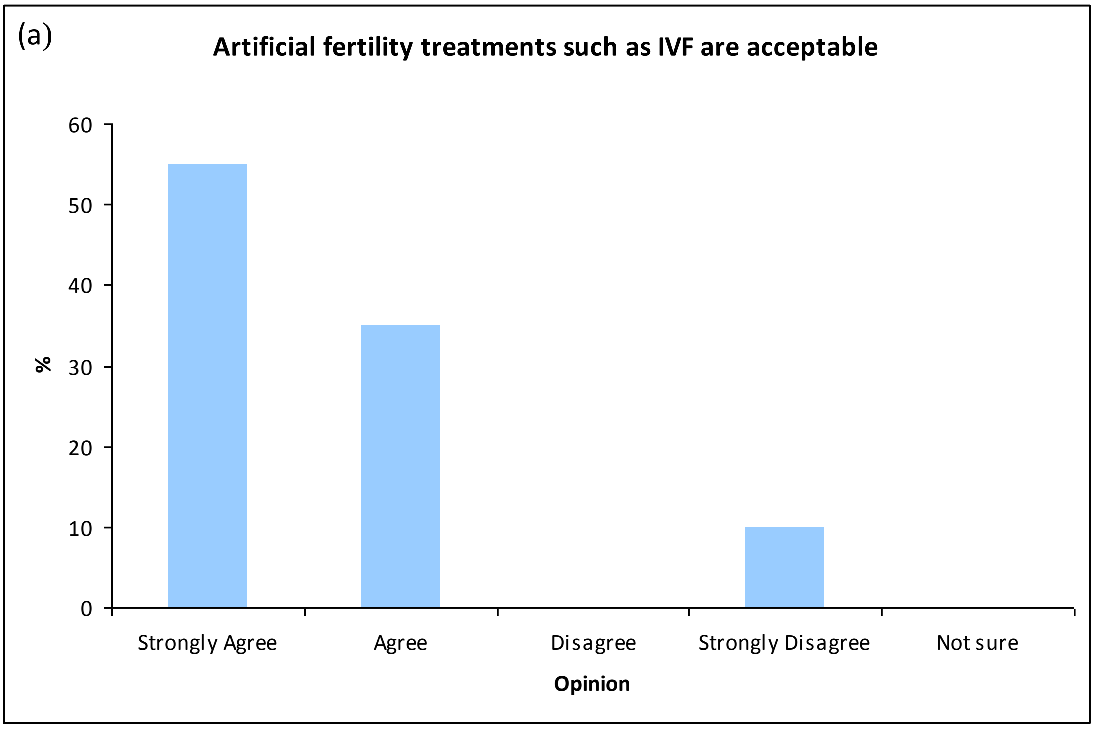
However, a concern not unreasonable is the possibility of health problems in the offspring. One may observe, for example, that embryos produced from ova, regardless of the genetic modification that has taken place, are less efficient in the formation of extra-embryonic structures and therefore become deficient in nutrients and oxygen, or that embryos produced from spermatozoa result in deformation of the embryo proper. Supposing that the embryos developed into foetuses and were then carried to full-term, it would still be very difficult to forecast potential health problems. Genetic disease and mutation can have a devastating effect on a person's quality of life: those with Duchenne muscular dystrophy experience progressive muscle weakness until they are unable to walk by the end of their childhood; those with Cystic Fibrosis only live until thirty-eight on average, requiring physiotherapy daily, and those born with Tay-Sachs disease become deaf, blind, paralysed and, usually by the age of four, dead. If genetic modification goes wrong, it could have disastrous effects on the well-being of the child. Then, supposing that the child does then mature without complication, would they themselves be able to reproduce? Modification of their genetic material could potentially result in infertility, raising the ethical issue concerning whether it is moral compromise one person's fertility for another's. There could also be social disadvantages to the offspring; would they be accepted by society, or discriminated against and bullied?

Finally, alarm arises at the prospect of reproduction without use for one of the genders, conjuring images of an ominous world in which one sex is eliminated. It can not be said that we ourselves do not have any control over the balance between males of females; the prevalence of males in China (119 males for every 100 females) illustrates that society can indeed influence the composition of a population. If bi-parental parthenogenesis was to become readily available, it would probably primarily be offered to female couples, eliminating the need for either surrogacy or extra-corporeal pregnancy. However, neither woman would be able to contribute a Y chromosome, dictating that every embryo must be female, and thus potentially agitating the structure of our population.

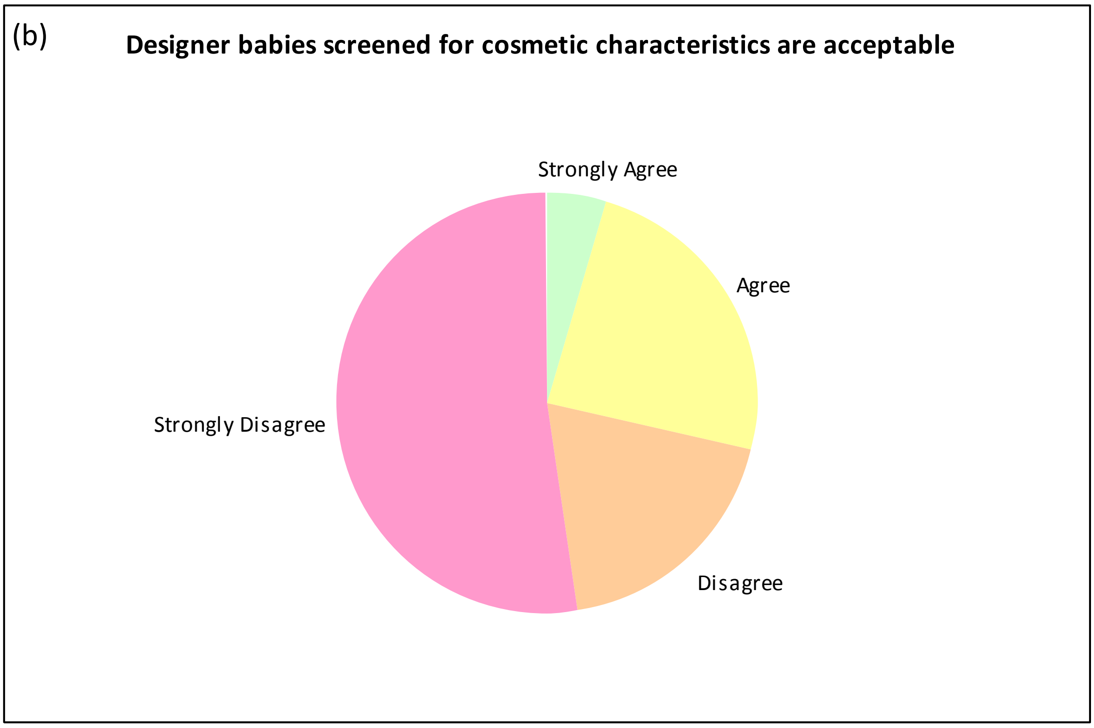
Whilst the disadvantages have been highlighted, they may not invariably outweigh the advantages. Although on the large scale, one may consider it an unnatural and costly procedure, focusing on the lives of individual people would be a different story. One may ask, why not just adopt? While many may be satisfied with this option, there are emotional disadvantages which could be overcome by bi-parental parthenogenesis. Firstly, there is the sense of identity that accompanies looking alike, which reinforces the feeling of belonging within a family. Secondly, there is the question concerning what defines motherhood; carrying the baby during gestation, genetic relation or providing the environment in which they grow up. Adoption can only tick one of these boxes. Thirdly, bi-parental parthenogenesis would spare the potential friction between family members arising where the child wishes to contact their 'real' parents. An alternative to adoption, allowing one of the couple to donate genetic material, would be the use of surrogacy and/or gamete donors. However, this raises another issue; there would be great difficulty in deciding which of the couple's gametes should be used to conceive and, thereafter, the individual with no contribution to their child's genetics may feel excluded.

Furthermore, one could argue that if bi-parental parthenogenesis was possible, preventing the public from accessing such technology could be interpreted as discrimination. As a modern society enthusiastic about tolerance, the way to access true equality would be through such technological developments. We have learnt to accept other reproductive technologies such as IVF, which has brought the world over 3 million 'miracle babies'. The technology of genetic modification could also be applied in the prevention of Angelman and Prader-Willi syndrome, further increasing the number of people the procedure would be of value to.

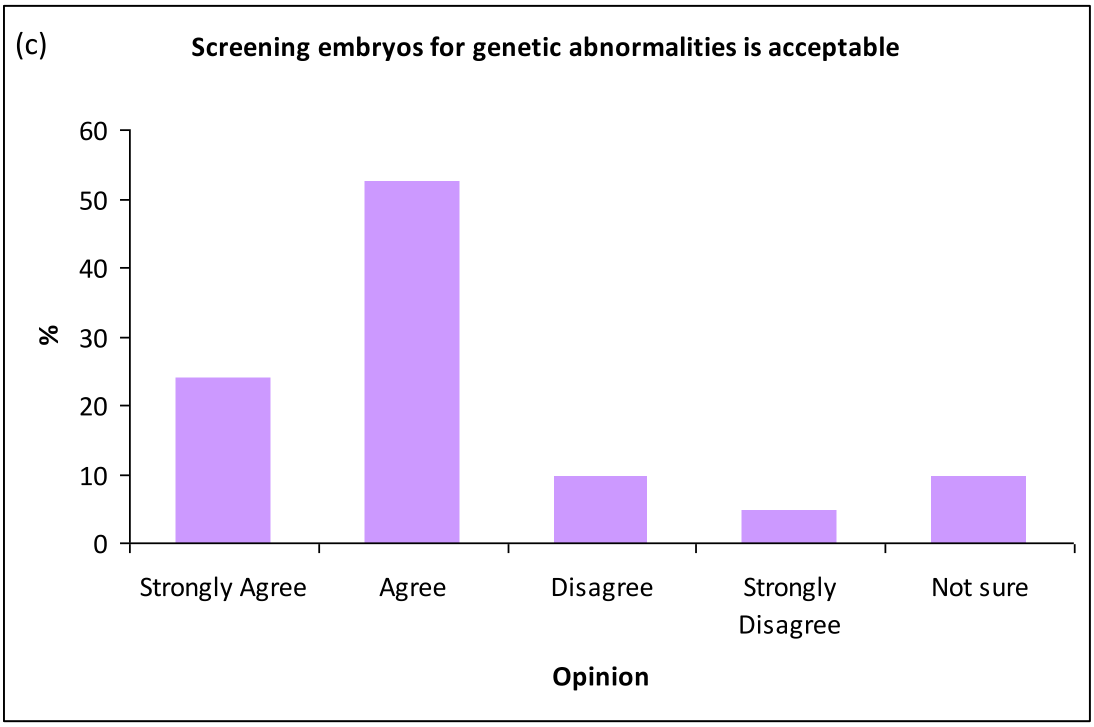
Curious about which side of the argument more people would support, I carried out a survey completed by 21 individuals. Whilst the sample size was very small, the results offer some insight into how the public would react to such developments by extrapolating from their opinions regarding current technology. The ages of participants ranged from fourteen to in their forties, living in regions including Gloucestershire and Cumberland.

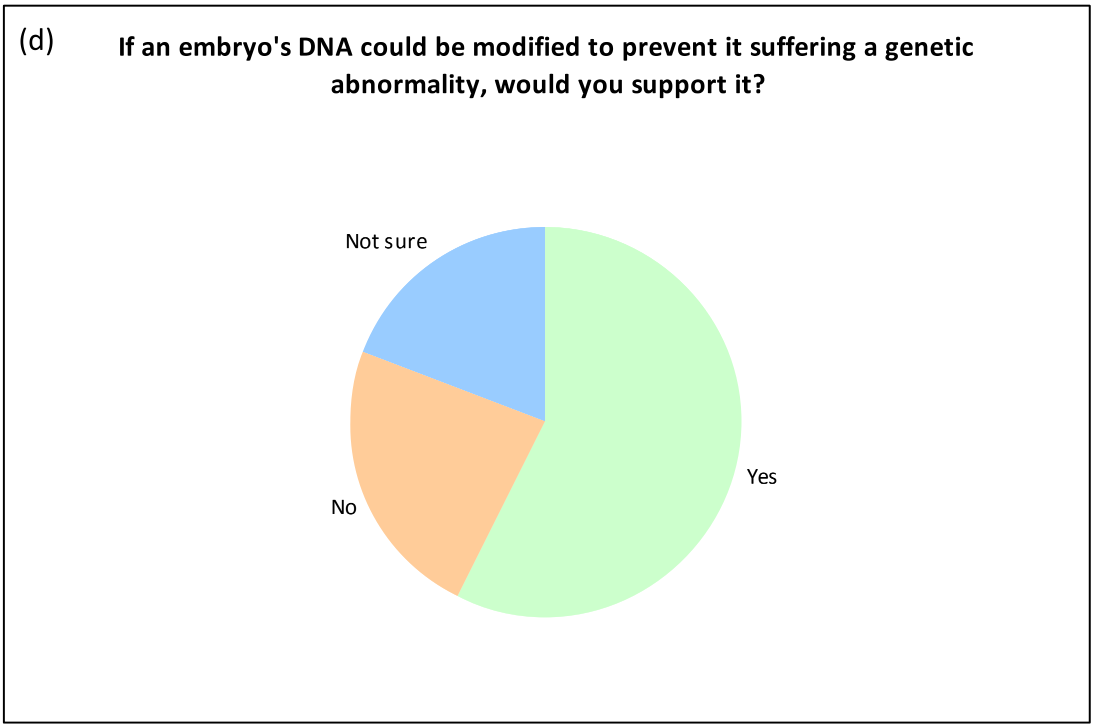


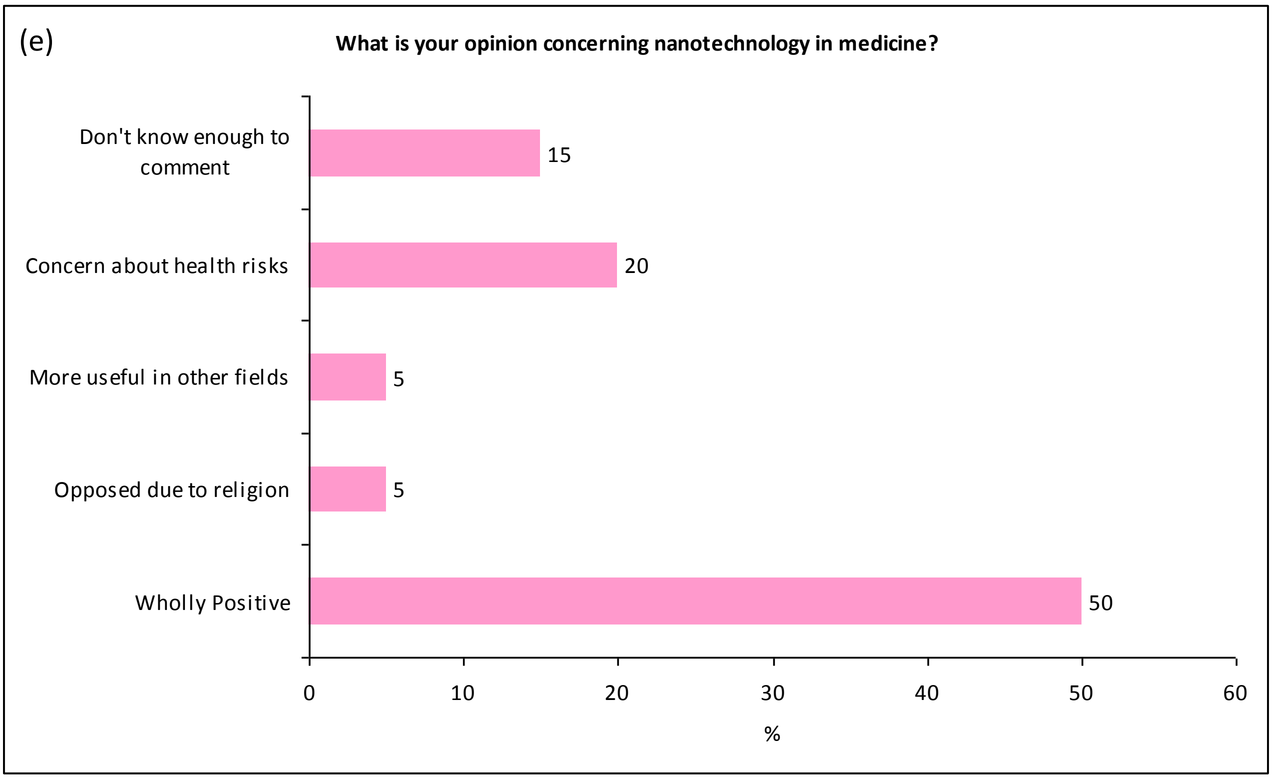
A total of 90% of those who participated believed that artificial fertility treatments are indeed acceptable. This suggests a general trend of open-mindedness towards reproductive technology, and would hopefully indicate willingness to, with time, accept new developments in this area. Not one person answering the question was unsure or weakly disagreed, alluding to the strong opinions and controversy surrounding the topic.



When the idea of IVF is extended to designer babies, 52.4% strongly disagreed whilst 19.0% disagreed. Strong opposition to screening embryos for cosmetic characteristics demonstrated by the modal opinion could, on one hand, indicate that when we try to control genetics it is immoral, suggesting that bi-parental parthenogenesis in its modification of genotypes to generate an outcome in the phenotype would evoke controversy. However, the strong reaction may be dependent on the distinction that the embryos are screened for cosmetic characteristics, which contradicts the politically correct ideas that appearance does not matter. This is supported by the following two (c and d) graphs, which have a more positive skew. In contrast, 76.2% believed screening for genetic abnormalities rather than aesthetics is acceptable (Graph c), suggesting that support for the applications of nanotechnology in genetic modification would be existent. However, when the idea of genetic modification rather than selection is introduced (Graph d), support dropped to 57.1%, but still represented the modal group. When asked about their motive for their decision, half (47.6%) of the participants would support DNA modification on the basis of offering the affected person a better quality of life. However, of sixteen comments, two applicants were doubtful due to plausible effects on the population, and two were opposed on the grounds of religious belief.

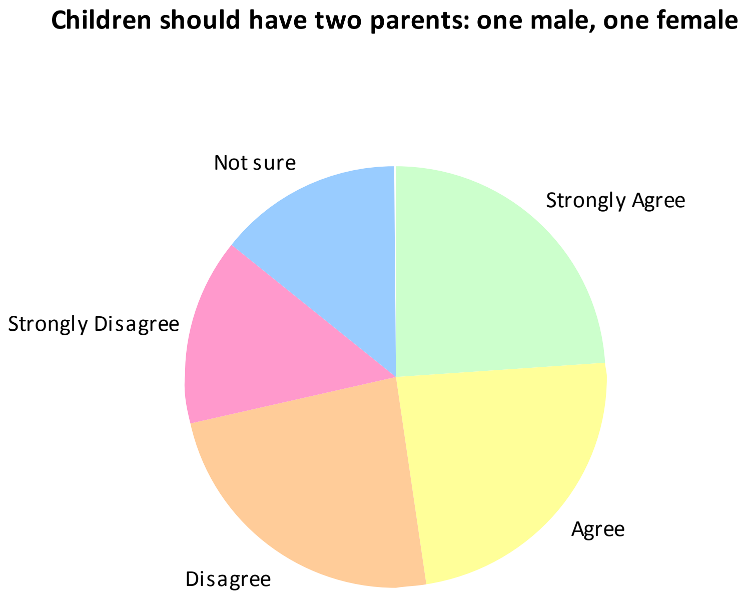




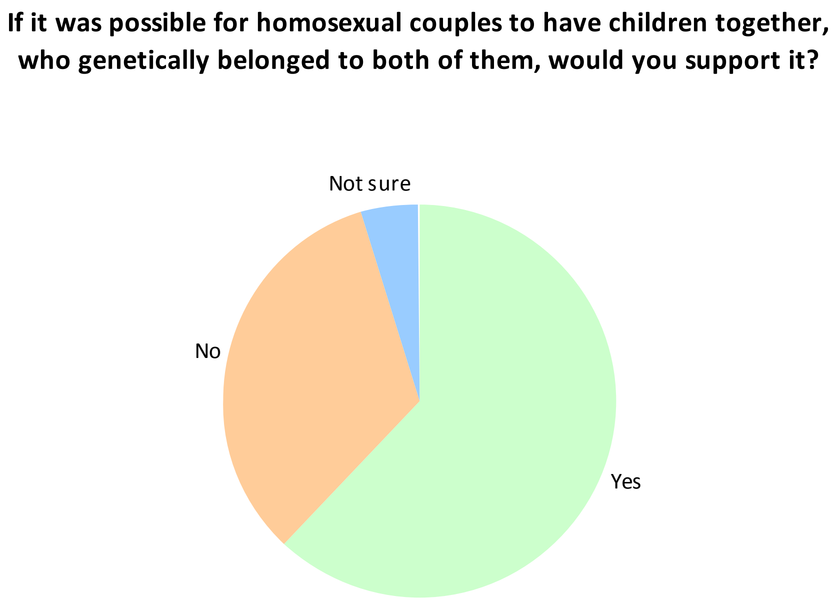


Assessing the view of the means of which the process would be carried out, the result was relatively positive. 85% of respondents had at least one positive thing to say about nanotechnology, ranging from "genius idea" "a very useful thing" to "amazing". Only one respondent was exclusively opposed, referring to the technology as "evil".

(f)



Graph (e) gives very little hint to whether reversing genomic imprinting and allowing bi-parental parthenogenesis to occur would have support; it appears that near equal numbers agree and disagree with non-nuclear families. Graph (f), however, shows a much larger modal group with 61.9% non-ambiguously agreeing with the prospect of allowing same-sex couples to procreate; however, a significant 1/3 of the participants still opposed the idea. Of 16 responses when asked for a justification for their choice, 8 supported the idea because it would increase equality; 3 supported the idea just because there was no reason not to; 3 opposed the idea due to their religious beliefs and 3 were concerned about the sociological effects.



Conclusion

In conclusion, bi-parental parthenogenesis could potentially take place in humans by the removal of methyl groups from imprinted genes using a nano-manipulator and then insertion of the modified genes into ova by use of micro-injection, liposomes or electroporation. However, in the writing of this paper I have made the assumption that methylation of cytosine is the only form of imprinting, whilst histone modification also has an effect, and that all imprinted genes are sexually antagonistic with little consequence resultant of the elimination of all imprinting. Asking Martin Christlieb of The Gray Institute, Oxford University, about the possibility of artificial demethylation, I was told that whilst it would theoretically be possible, interfering with DNA is very complex. It can also be seen that there are numerous ethical issues associated with bi parental parthenogenesis which could inhibit its development.

References

-A Short History of Nanotechnology www.foresight.org/nano/history.html
-Booker, R.B., Boysen, E.B, (2005), *Nanotechnology for Dummies*, Indianapolis, Wiley Publishing, Inc
-Liposomes http://www.dadairs.com/liposomes.htm
-Phospholipid http://www.britannica.com/EBchecked/topic/457489/phospholipid
-Liposomal doxorubicin http://www.macmillan.org.uk/Cancerinformation/Cancertreatment/Treatmenttypes/Chemotherapy/Individualdrugs/Liposomaldoxorubicin.aspx
-Stealth liposomes: review of the basic science, rationale, and clinical applications, existing and potential http://www.ncbi.nlm.nih.gov/pmc/articles/PMC2426795/
-Microarrays: Chipping away at the mysteries of science and medicine http://www.ncbi.nlm.nih.gov/About/primer/microarrays.html
-DNA Microarray Methodology http://www.bio.davidson.edu/courses/genomics/chip/chip.html
-Pharmacogenomics http://www.ornl.gov/sci/techresources/Human_Genome/medicine/pharma.shtml
-Oxford Nanopore technologies http://www.nanoporetech.com/sections/first/1
-AFM operation http://www.chemistry.uoguelph.ca/educmat/chm729/afm/operate.htm
-Atomic Force Microscopy http://www.nanoscience.com/education/afm.html
-Manipulation of DNA- rupture forces http://www.3rdtech.com/dna.htm
-The Nano-Manipulator http://www.warrenrobinett.com/nano/index.html
-Size of carbon in several environments http://www.webelements.com/carbon/atom_sizes.html
-Size of hydrogen in several environments http://www.webelements.com/hydrogen/atom_sizes.html
-Nanomanipulation by atomic force microscopy http://www.nanobiomat.de/pdf/RubioResearchNewsPreprint.pdf

-Wolpert, L.W., (1991), *Triumph of the embryo*, USA, Oxford University Press

-Female sharks can reproduce alone, Researchers find http://www.washingtonpost.com/wp-+dyn/content/article/2007/05/22/AR2007052201405.html
-Parthenogenesis http://www.encyclopedia.com/topic/parthenogenesis.aspx
-Asexual Stem Cell Production http://www.accessexcellence.org/WN/SU/parthenogenesis.php
-Activation of the unfertilised egg by Ultra-Violet rays http://www.sciencemag.org/content/40/1036/680
-The parthenogenetic development of rabbit oocytes after repetitive pulsatile electrical stimulation http://www.ncbi.nlm.nih.gov/pubmed/2209460
-Birth of parthenogenetic mice that can develop to adult hood http://www.ncbi.nlm.nih.gov/pubmed/15103378
-Mice created without fathers http://news.bbc.co.uk/1/hi/sci/tech/3643847.stm
-Jones, S.J., (2000), *The language of the genes*, London, Harper Collins
-Wills, C.W., (1991), *Exons, Introns and talking genes*, Oxford, Oxford University Press
-What is Prader-Willi Syndrome? http://pwsa.co.uk/main.php?catagory=1
-Diagnosis of Angelman Syndrome http://www.angelmanuk.org/
-Ridley, M.R., (1999), *Genome*, London, Fourth Estate
-Silent Struggle: A new theory of pregnancy http://www.nytimes.com/2006/03/14/health/14preg.html?_r=1&pagewanted=all-
-Association of H19 Promoter Methylation with the Expression of H19 and IGF-II Genes in Adrenocortical Tumours http://jcem.endojournals.org/cgi/content/abstract/87/3/1170
-DNA Methylation and Genome Stability http://www-medchem.ch.cam.ac.uk/lab_rotations/murrell.php
-Mechanisms of mono allelic gene expression in mammals http://www.nature.com/horizon/epigenetics/kq/2_Reik.html

-Mechanisms of mono allelic gene expression in mammals
http://www.nature.com/horizon/epigenetics/kq/2_Reik.html
-Initial sequencing and analysis of the human genome
http://www.nature.com/nature/journal/v409/n6822/full/409860a0.html
-Herdewijn, P.H., (2005) *Oligonucleotide synthesis*, Totowa, Humana Press
-Positions of genes Inside the Cell Nucleus Exert Biological effects
http://www.scientificamerican.com/article.cfm?id=gene-location-affects-expression&page=2
-Chromosomes FAQs
http://www.ornl.gov/sci/techresources/Human_Genome/posters/chromosome/faqs.shtml
-Analysis of DNA methylation using bisulphite sequencing
http://www.methods.info/Methods/DNA_methylation/Bisulphite_sequencing2.html
-Molecular tool: Electroporation
http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/McCord/electroporation.htm

-History of Human Genetic and Reproductive technologies
http://www.geneticsandsociety.org/article.php?id=3157
-CIA- The World Factbook https://www.cia.gov/library/publications/the-world-factbook/index.html
-The Religious Response to reproductive technology http://www.religion-online.org/showarticle.asp?title=807
-Ball, P.B., (2011), *It's alive, I tell you!*, In *NewScientist*, 2799, 30-31
-Happy Families: The non-nuclear options http://www.independent.co.uk/news/uk/home-news/happy-families-the-nonnuclear-options-1690129.html
-Census 2001- Families of England and Wales
http://www.statistics.gov.uk/census2001/profiles/commentaries/family.asp
-Information on Adoption Law http://www.adoption.org.uk/information/adoption_law.html
-What is Cystic Fibrosis? http://www.cftrust.org.uk/aboutcf/whatiscf/
-Duchenne Muscular Dystrophy http://www.muscular-dystrophy.org/about_muscular_dystrophy/conditions/97_duchenne_muscular_dystrophy
-NINDS Tay-Sachs Disease Information Page
http://www.ninds.nih.gov/disorders/taysachs/taysachs.htm
-China faces growing gender imbalance http://news.bbc.co.uk/1/hi/world/asia-pacific/8451289.stm
-More than 3m babies born from IVF http://news.bbc.co.uk/1/hi/health/5101684.stm