Synthetic DNA and mitochondrial donation: no need for donor eggs?

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ABSTRACT

Mitochondrial replacement therapy has been developed in order to prevent the transmission of mitochondrial mutations, yet it raises ethical concerns, particularly regarding the involvement of third-party DNA and the risks associated with donor procedures. This paper explores an alternative approach using synthetic DNA (synDNA) to construct mitochondrial organelles, thereby bypassing the need for donor oocytes and bypassing risks to donors. We argue that those who support mitochondrial replacement techniques as an ethically acceptable means of preventing the transmission of mitochondrial disease should consider the use of synthetic mitochondria as a preferable ethical alternative, should it prove technically viable. That this will be viable is more than we can demonstrate here. However, progress in synDNA technology suggests that it is not unreasonable to think that synthetic mitochondria creation is feasible, and perhaps even probable.

INTRODUCTION

Mitochondrial replacement therapy (MRT) is designed to prevent the transmission of mitochondrial mutations by replacing the prospective mother's mitochondria with mitochondria obtained from a donor.[1–3] This ensures that women with mitochondrial disease can reproduce, while reducing the risk that their offspring will suffer from mitochondrial disease.

Several ethical concerns have been raised in relation to MRT. Some have worried that since MRT involves the genetic input of three people, instead of two as in conventional assisted reproductive technologies (ARTs), it would effectively create offspring with three parents.[4 5] While this prospect has received widespread media attention, in the ethics literature there has been more interest in ethical concerns regarding the alteration of the human germline, and the consequences that this might have on future generations.[6] Some bioethicists worry about the safety of the procedure, or the potential for unforeseen genetic and health issues for offspring.[7] Others have noted that in order to facilitate one woman's aim of transmitting nuclear DNA, another woman has to undergo the invasive procedures of ovarian stimulation and egg harvesting.[8] But another feature of MRT is the need to perform an intervention on two eggs in order to obtain one that fulfils the requirements of the prospective parents.

The purpose of MRT is premised on the desirability of not transmitting faulty mitochondria from mother to child. It is not the intention of either the intended parents or the healthcare team that the mitochondrial DNA (mtDNA) donor would be treated as a parent. The law treats mitochondrial donors as strangers whose identity and motivations are of as little significance as the motivations of those who donate blood.[9] But the incorporation of third-party DNA—even though it is 'merely' mitochondrial—may open the way for relationship claims in the future.[10–13] The case of gamete donation is potentially instructive here, showing that the law can change. For many years, both prospective parents and gamete donors had been encouraged to see gamete donation as a one-off event unlikely to be of any significant interest to the offspring. This has proved false, and anonymity has been removed in many legislatures, sometimes retroactively. Accordingly, those who use MRT today may also be subjected to fluctuations in legal and social norms that are beyond their power to predict or control. We cannot assume, either, that offspring will regard their mitochondrial donor as having no special significance to them; as with offspring born following gamete donation, we simply do not know how they may feel.

There may be an alternative to mitochondrial donation that avoids invasive treatment for donors and bypasses the creation of genetic relationships between the offspring and any third party. In this paper, we will consider the use of synthetic DNA (synDNA)—that is, DNA created wholly in the laboratory from its bare molecular ingredients—to construct mitochondrial organelles to replace the mutated mitochondria carried by the prospective mother. In our proposed approach, synthetic mtDNA would be introduced into mitochondria in situ, within the egg cell, after the removal of pre-existing mtDNA, as described in previous studies.[14–16] This process occurs in culture, avoiding the need to extract and reintroduce mitochondria. We consider whether such a technique would be preferable to the use of mitochondria donated by a third party.

Before we discuss this possibility, it is important to note that we do not, in this paper, undertake a critical analysis of the basis for undertaking MRT in general. MRT has been criticised by several ethicists on a number of grounds.[17–19] However, our aim here is simply to show that the use of synDNA offers an equally acceptable or even morally preferable way of achieving the reproductive goals of women with mitochondrial disease. It might also be of interest to lesbian couples aiming to undergo ART in order to have children related to both partners, and perhaps to women whose oocytes are damaged by ageing and could be 'rejuvenated' with the help of synDNA technology.[20]

Creating synDNA

DNA synthesis involves assembling molecules (adenine, thymine, guanine and cytosine) into long chains. Over the past two decades, researchers have

developed *in vitro* methods for DNA synthesis via chemical reactions. This enables the construction of DNA sequences outside of a cell, in a machine in the lab. These synthetic or printable DNA molecules offer the possibility of generating specific DNA sequences from scratch. While current synthesis techniques face challenges such as cost, time and sequence length limitations, recent advances have substantially improved efficiency, addressing these constraints. Moreover, that something is too technologically challenging to be done (or done easily) today does not mean that it will be too challenging tomorrow, and so it is worth thinking about the ethics in advance.

In 2007, the team led by Craig Venter achieved a milestone by synthesising and transplanting an entire artificial genome.[21] In this study, researchers completely replaced the genome of the bacteria *Mycoplasma capricolum* with a synthetic version of another bacterial genome (*Mycoplasma mycoides*). The resulting bacteria exhibited behaviours consistent with *M. mycoides*, confirming the expected phenotype from the artificial genome. Some years later, Venter and his team undertook the task of redesigning the entire genome of *M. mycoides*, removing genes deemed unnecessary for bacterial survival in laboratory conditions, and generating the first genome entirely redesigned using a computer.[22] Advances in synDNA techniques have also been achieved in eukaryotic organisms.[23] In 2023, it was shown that half of the genome of the yeast can be synthetically manufactured and replaced in wild-type yeast.[24] The same year, this group also built a novel extra chromosome that does not exist in the wild strain.[25] Additionally, the group was able to use synDNA techniques to rebuild chromosomes from yeast with structural alterations in order to get insights into the function of different genomic regions in yeast.[26 27]

The entire genome of *M. mycoides* consists of approximately 1.2 million nucleotides,[28] while the yeast genome contains far fewer nucleotides than the human (12 million,[29] as compared with approximately 200 million). While current techniques do not yet permit the construction of human chromosome-sized strands of DNA, it is already possible to engineer certain human sequences. For instance, mtDNA consists of 16.569 nucleotides. This is far less than the 1.2 million nucleotides of synDNA generated for *M. mycoides* or the 6 million nucleotides generated for yeast. On the surface, then, building synthetic human mitochondria ought to be well within current technological capabilities. Indeed, several papers in the scientific literature describe techniques to introduce exogenous DNA into mitochondria[30–32] and even remove pre-existing mtDNA.[14–16]

Hence, it is feasible to imagine a scenario in which synthetic mitochondria could be engineered by first removing pre-existing mtDNA, as described in the literature,[14–16] and then introducing exogenous synDNA[21 23 30 32] (figure 1). The removal of endogenous mtDNA could be accomplished using nucleases[32]—enzymes

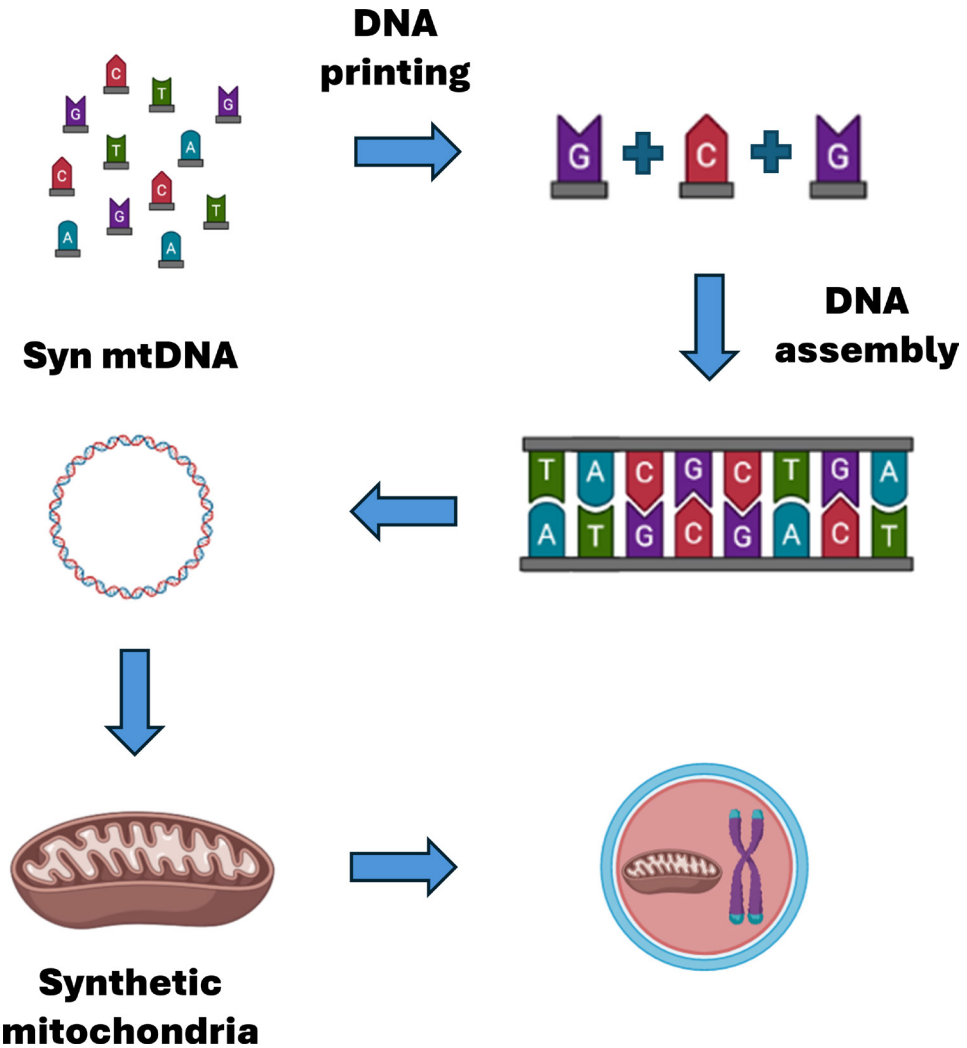

Figure 1 Process of DNA synthesis in order to create synthetic mtDNA molecules to be transferred to mitochondria and generate eggs out of synMRT. mtDNA, mitochondrial DNA; synDNA, synthetic DNA.

that cleave DNA—or by suppressing mitochondrial gene expression with morpholinos,[30] as previously demonstrated. The resulting DNA-depleted mitochondria could then be loaded with exogenous synDNA through established delivery methods, such as electroporation (a technique that uses electrical pulses to transiently open membrane pores)[16] or via mitochondria-targeted peptide carriers,[15] enabling genome replacement without donor mitochondria. Techniques such as electroporation may become obsolete in a few years, and new methods may arise. What is important for the purposes of our paper is that previous authors have already (1) removed DNA from mitochondria and (2) inserted DNA into mitochondria, so it is a feasible technique. This would be similar to MRT but without requiring third-party mitochondria. We term this 'synMRT' to distinguish this technique from the conventional one. Absent any evidence to the contrary, we assume here that this technique is at least as safe as MRT techniques already in use.

Avoiding harm

The process of oocyte retrieval requires hormonal stimulation to induce the maturation of multiple eggs within the ovaries, followed by a surgical procedure to extract the eggs. In conventional MRT, this process of oocyte retrieval is carried out both on the prospective mother and on the mitochondrial donor. Oocyte retrieval is moderately invasive and poses risks. One of the most severe immediate risks is ovarian hyperstimulation syndrome, which in rare cases has led to death. Consent protocols for oocyte retrieval emphasise that donors should carefully consider the physical and emotional aspects of the process.[33] From the perspective of avoiding harm, it seems evident that a novel technique with similar outcomes to the current ones, but fewer risks, should be preferable; and synMRT does seem to offer this opportunity.

If synDNA could be used, it would offer a clear advantage, since only the prospective mother would be exposed to the risks and discomfort of oocyte retrieval.

There may be concerns about risks to the offspring, as well as ongoing risks that arise through the transmission of the synthetic mitochondrial DNA to future generations. This might well be a concern if we imagine that scientists are devising new forms of mtDNA that diverge from previously known variants. However, in practice, there is no reason why mtDNA would need to be designed *de novo*. Instead, it could be made to match, nucleotide for nucleotide, either the mitochondria of the prospective mother (minus the mutation), or of some other person who is known to be healthy. In this way, the synthetic mitochondria will pass to the offspring but will not involve any new genetic form, but a preexisting variant shared by millions of people.

Since the synthetic mitochondria would carry the same information as other mtDNA, like the ones used in MRT, the genetic risks involved appear fairly minimal. That is, there is no reason to think that errors in DNA transcription would occur, or that unknown variants would be used. If there were risks, therefore, they would not be connected with the DNA itself, but with the methods by which the DNA is produced. Synthetic mtDNA would be created in a laboratory: it might be infected with contaminants, or its functioning might be affected in unforeseen ways because of the unusual nature of its origins. Mistakes could be made. Undoubtedly, this is something to think about in the context of synMRT. However, the possibility of error or contamination represents problems *for* the technique, not *with* it; it tells us that it ought to be pursued with care, not that it ought not to be pursued at all.

Additionally, it is worth noting that existing MRT procedures also involve interference with natural processes in ways that may carry unknown implications both for future offspring and future generations.[34] Yet it is not obvious that the concerns in one case refer to anything qualitatively different from the concerns in the other; hence those who believe MRT is acceptable ought probably to regard the use of synDNA in the same situations as acceptable.

Third parties

A second issue raised by the use of current MRT techniques is the involvement of a third party in the reproductive project of the prospective parents. Offspring born following conventional MRT inherit genetic material from individuals with whom they may not have a direct familial relationship. It is interesting to note that the advent of MRT has fuelled strong arguments against the genetic conception of reproduction, further calling into question the degree to which the parent–child relationship is essentially a genetic one.[35]

However, for many people, genes do indeed play an important part in their reproductive and parenting aspirations, even if bioethicists tend to be critical of this. So although the assumption that genes are the essence of parenthood, or reproduction, has been subject to criticism in the literature,[36 37] this is not necessarily mirrored in the fertility industry, nor in society generally. MRT is based on the idea that genetic reproduction is such a fundamentally important thing that it justifies the extraordinary technological, invasive and legally complex medical machinery being used to perpetuate it. For the purposes of this paper, we do not take a stance on this debate. However, it is worth noting that it does not matter whether people are mistaken to place weight on genetic relationships. What matters is that such beliefs and values may be at the root of suffering that arises when people feel compelled to 'allow' third party genetic material into their reproductive projects. And, as we noted, while the law currently treats mitochondrial donors as strangers with no particular claims or responsibilities in respect of a child born through MRT, legal conventions can and do change.

But all these problems could be circumvented through the use of synthetic mitochondria. The basic idea would be that, instead of 'importing' mitochondrial DNA from a third party, it would be possible to build new mitochondria *de novo*. The relatively small size of the mitochondrial genome makes this a not-wholly-implausible turn of events. And if used, this would avoid having to worry about emotional or even legal ties to a gene-donor at some point in the future, because there would be no such donor. Accordingly, if an alternative to standard MRT becomes available that enables patients to avoid recourse to third-party donors, it seems reasonable to view this as a desirable step. People who push for such a step may display a degree of genetic essentialism, but no less so than the parents involved in standard MRT, or indeed many other forms of fertility treatment.

Maybe we are moving too quickly here. If synDNA is used to create mitochondria, there is a question to be asked about the origin of this synDNA, since presumably there would be some person whose 'healthy' mitochondria provided the template on which the synthetic mitochondria were modelled. One might argue that the third-party parenthood issue would in fact remain if the synDNA exactly replicates the mitochondria of another person. However, unlike nuclear DNA, which is (apart from identical twins) linked to a unique individual, mtDNA is inherited maternally and is specific to a maternal lineage rather than an individual. This means that only a few versions of mtDNA

exist in the human population, and each is shared by many thousands, perhaps millions, of people.

In this sense, mitochondrial DNA is more like a blood type than a genome. This undermines the idea that there is a special relationship with those who share one's mtDNA or that one has a right to know them or form relationships with them. On the other hand, people who share mtDNA have not ordinarily made the decision to have their eggs harvested, with all the costs and risks that this entails. They have taken no deliberate causal role in the process of the coming-into-being of all those who share their mtDNA. In this, we can see a difference between the mtDNA donor and the person who simply happens to share the same mtDNA with the offspring but has taken no active steps to bring about this link.

This discussion has some resonance with the debate as to what parenthood status inheres in. A conventional mtDNA donor may in fact be significant in the narrative of a child's coming-to-be story because of her actions and choices. In the case of synMRT, we circumvent the need for a person to donate their mtDNA. Thus, whether one takes genetic accounts or causal accounts of parenthood to be significant, synMRT offers prospective parents a way to avoid the inclusion of others into their parenting project, and thereby to bring their project more into line with conventional parenthood.

Mitochondrial enhancement

SynMRT could also be used to design other variants for some mitochondrial genes. If we look more closely at the content of mtDNA, there are a total of 37 genes. Among these, 13 are coding genes, meaning they provide instructions for the synthesis of proteins essential for oxidative phosphorylation, the process by which cells generate energy. These coding genes include subunits of the respiratory chain complexes I, III, IV and V, crucial for mitochondrial protein synthesis. The remaining 24 genes in mtDNA are non-coding genes, comprising 22 tRNAs (transfer RNAs) and 2 rRNAs (ribosomal RNAs), which play key roles in the translation of the genetic code into functional proteins within the mitochondria. Some studies have suggested that certain mitochondrial gene variants may be linked to variations in endurance capacity. This opens the possibility of what might be termed 'mitochondrial enhancement', where mitochondrial alterations are undertaken to improve athletic performance, for example. To date, there is no consensus as to whether this will ultimately be possible.[38–41] This offers an interesting prospect for further research, not least because it seems to undermine the claim that mitochondria are insignificant in terms of their phenotypic effects.

CONCLUSIONS

In this paper, we argue that if the techniques developed in order to synthesise DNA fulfil their potential, they would offer a relatively straightforward path towards the creation of mtDNA. We ask whether the use of synMRT would be ethically preferable to MRT. We suggest that synMRT would enable the creation and transfer of mtDNA without having to retrieve oocytes from a donor. Only one woman, the prospective mother, would need to undergo oocyte retrieval surgery if synMRT were used. Moreover, the creation and use of synDNA in MRT would enable prospective parents to feel confident that they are the only people who have contributed to their offspring's genetic makeup. There might be risks involved in the creation, storage and transfer of synMRT, but it is not obvious that these risks are greater than those involved in conventional MRT.

If proven safe and effective, we suggest that those who currently accept MRT as an ethically sound means of avoiding mitochondrial disease, while allowing women to transmit nuclear DNA to their offspring, should regard synMRT as an ethically preferable means of achieving these same ends.

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