Several limitations could prevent the use of embryoids for most immediate research and therapeutic questions, however. The failure rate is very high – the groups estimate that less than 1% of starting cell clusters successfully develop into embryoids. Tarazi *et al.* showed that some mESC lines produce embryoids that could not develop for more than six days in culture, indicating that embryoid development depends on the state of the starting cells. Furthermore, embryoids are highly variable in size and shape. Importantly, a lack of development past E8.5, noted by both groups, prevents investigation into the development of most organs and neural tissue.

Optimization of the three starting cell populations and culture conditions will be key to overcoming these limitations. For instance, embryoid culture media contain serum from the blood of rat and human umbilical cords; these sera are poorly characterized and can vary between batches, which might have a major effect on embryoid development. Changes to how carbon dioxide and oxygen are mixed, controlled and pressurized in the media, and tweaks to dynamic culture conditions, might allow longer *in vitro* growth, because these factors have been hypothesized to enable proper development[11,12]. Incorporation of other components, such as stem-cell-derived placental cell types, could extend embryoid development and reduce variability.

It is worth highlighting that these are not actual mouse embryos, but stem-cell-derived models of early mouse embryonic development. The use of terms such as 'synthetic embryos' for cell-based models of development has been generally discouraged by the International Society for Stem Cell Research (ISSCR)[13]. Consensus around a unified nomenclature is needed to accurately represent embryoid-based approaches to scientists and the public, as is currently being done for other cell-based models of development[14].

Eventually, researchers will want to apply these findings to human stem cells. The larger size of human embryos might mean that embryos depend more heavily than do mouse embryos on the placenta and vascular system – these properties could inherently limit *in vitro* development, which depends on diffusion for nutrient and gas exchange. Because human development takes several times as long to reach a stage equivalent to E8.5, generating human embryoids to late stages will be more costly and less feasible. And the use of human cells poses unique ethical challenges. The ISSCR has updated its guidelines to balance ethical concerns with the need for more research. For example, a '14-day rule' that limits *in vitro* studies of human embryos to the first two weeks of development has been replaced with oversight guidelines[10]. Specific consent for human stem cells to be used for embryoid research, and other legal issues, will need to be revisited before human-embryoid experiments can proceed.

The current studies elevate mouse stem-cell biology to the next level. The next frontiers – pushing the limits of *in vitro* development of mouse embryoids and applying these findings to human cells – are evident. Progress in these areas will undoubtedly advance our understanding of human embryonic development, improve our ability to generate disease-relevant cell types from stem cells and study the factors that affect pregnancy.

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Organic chemistry

A stable alternative to an explosive reaction

Vignesh Palani & Alison Wendlandt

The ozonolysis reaction is a classic of organic synthesis, but involves the formation of potentially explosive reaction intermediates. A modern, safer spin on this process makes use of previously overlooked chemistry. **See p.81**

In organic chemistry, a core set of fundamental mechanisms can be used to describe what happens in numerous individual reactions. But some reactions stand out because their mechanisms are unique. One such example is ozonolysis – an oxidation reaction widely used to cleave carbon–carbon double bonds. On page 81, Ruffoni *et al.*[1] present a modern riff on this mechanism, opening up a strategy for oxidative cleavage that overcomes the practical limitations of the classic ozonolysis reaction.

"The substitution of an oxygen for a nitrogen atom transforms a transiently formed, explosive species into an isolable intermediate."

Alkenes are the family of organic compounds that contain carbon–carbon double bonds (C=C bonds), and include many chemicals used as feedstocks for industrial processes, such as terpenes and fatty acids. Ozonolysis cuts alkenes into two, incorporating oxygen atoms into the resulting fragments. Discovered[2] in the 1840s, ozonolysis was widely used as an analytical tool for determining whether C=C bonds were present in organic molecules, and the position of such bonds in a molecule, before the advent of modern spectroscopic techniques.

Today, chemists use ozonolysis to convert alkenes into compounds that contain carbonyl (C=O) groups (Fig. 1a), thereby converting one group that has many uses in organic synthesis (the C=C bond) into another synthetically useful group. This invaluable transformation has few alternatives, and has been used in many important applications – including the syntheses of the antimalarial compound artemisinin and of the antibiotics ceftibuten and cefaclor[3].

One big limitation of ozonolysis is that the key reagent – ozone (O_3) – is a lethal, highly unstable and potent oxidizing agent. Ozone is generated in nature from molecular oxygen (O_2) during lightning strikes and through irradiation with ultraviolet radiation in the stratosphere. But it can't be stored in a bottle, because it rapidly decomposes back to O_2 under ambient conditions. A specialized

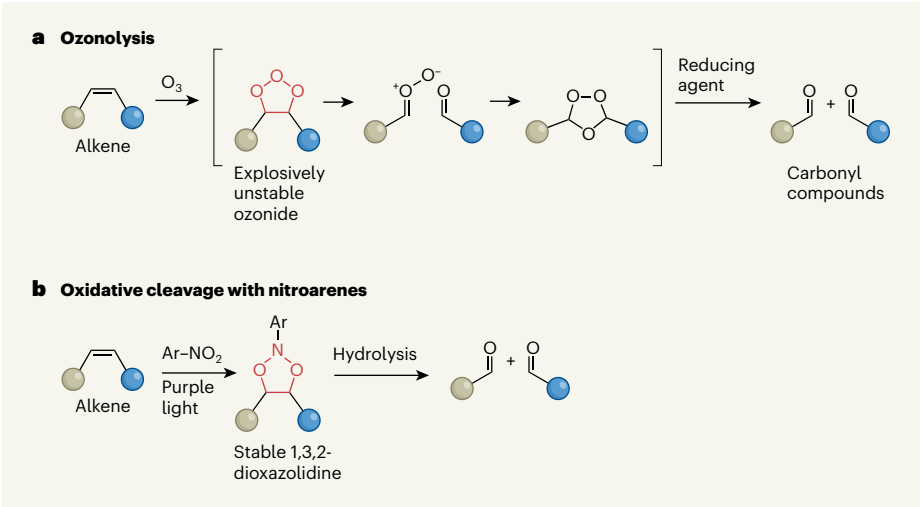

Figure 1 | Oxidative cleavage of alkenes without ozone. a, In the classic ozonolysis reaction, alkenes are cleaved across the carbon–carbon double bond by ozone (O_3) to produce carbonyl compounds as products. The first step is the formation of an unstable ozonide intermediate (core structure shown in red), which can be explosive. This undergoes a two-step rearrangement, whereupon treatment with a reducing agent produces the final products. Spheres represent any chemical group; square brackets indicate that the enclosed compounds are transient reaction intermediates. **b**, Ruffoni *et al.*[1] report an alternative reaction in which alkenes are reacted with compounds called aryl nitrates ($ArNO_2$; Ar represents a benzene ring, or analogues thereof). Irradiation with purple light excites the aryl nitrates, so that they can react with the alkenes to form stable intermediates called 1,3,2-dioxazolidines; these compounds are not explosive, even though their core structure (red) is highly similar to that of ozonides. Hydrolysis of these intermediates produces the carbonyl compounds. The overall process is safer and easier to carry out than ozonolysis.

apparatus, known as an ozonator, is needed to generate the gas in the laboratory, where it can be immediately bubbled into the reaction solution. However, many chemists do not have access to ozonators.

Furthermore, ozonolysis reactions proceed through potentially explosive intermediates (known as ozonides), some of which must be quenched in steps that produce a lot of heat, and which typically require the use of cryogenic cooling and other risk-mitigation techniques. Chemists can be trained to perform these reactions safely, but many do not receive such training because of the limited availability of ozonators. Moreover, further hazards can arise when ozonolysis is carried out at very large scales, and other limitations occur at very small scales.

For decades, chemists have tried to develop safer alternatives to ozonolysis, ideally maintaining the high selectivity with which the classic reaction forms products, and avoiding the formation of undesired oxidation products. Useful alternatives have been identified for certain types of molecule[4,5], but no reaction has succeeded in fully replacing ozonolysis to perform oxidative cleavage of alkenes in diverse synthetic contexts.

Ruffoni *et al.* have now identified reaction conditions that enable the desired alkene-to-carbonyl transformation through a mechanism that parallels that of ozonolysis, but without the need for ozone (Fig. 1b). This protocol presents a safe and practical alternative that avoids the formation of undesired oxidation products, boasts high selectivity in the formation of alkene-cleavage products and works reliably with a wide range of alkenes.

Instead of ozone, Ruffoni and colleagues' reaction uses nitroarenes — a readily available class of organic compound — to cleave alkenes. Irradiation of a starting nitroarene with purple light generates an excited electronic state of the compound, which intercepts the alkene to produce intermediates known as 1,3,2-dioxazolidines. Subsequent hydrolysis fragments these intermediates in a controlled way, producing the final carbonyl-containing products. Notably, the initial reaction of the nitroarene is not favoured when the reaction is simply heated. Instead, energy harnessed from photons is required for this reaction to occur.

Unlike the explosively unstable ozonide intermediates produced during ozonolysis, the analogous 1,3,2-dioxazolidines are stable enough to be isolated. Some can even be stored in the solid state for a few days at room temperature, or for longer periods at −30 °C. The key difference between ozonides and 1,3,2-dioxazolidines is that an oxygen atom in the former is replaced by a nitrogen atom — for this reason, Ruffoni *et al.* describe 1,3-2-dioxazolidines as *N*-doped ozonides. It is fascinating to note that the seemingly simple substitution of an oxygen for a nitrogen atom transforms a transiently formed, explosive species into an isolable intermediate.

The innate reactivity of light-activated nitroarenes, and the reaction of these compounds with alkenes, had been reported decades ago[6,7]. However, the untapped potential of this once-obscure transformation was long unrecognized, and Ruffoni *et al.* had to undertake substantial research before they could make the fragmentation step in their protocol robust enough for general use. The authors' fully developed method makes it possible to cleave C=C double bonds in a wide array of organic molecules that contain a variety of other chemical groups, using conditions that avoid the formation of explosive intermediates. Another advantage of this method is that the size and electronic properties of the nitroarenes can be fine-tuned, so that a specific C=C bond can be targeted in molecules that contain more than one such bond. Such fine-tuning is not possible in ozonolysis reactions.

In addition to providing a user-friendly alternative to ozonolysis, the new findings might inspire further development of reactions that harness the reactivity of nitroarenes. Indeed, a similar protocol for the oxidative cleavage of alkenes using nitroarenes as oxygen-transfer agents was recently reported[8] by a group working independently of Ruffoni and colleagues. The parallel development of this method reveals a growing interest in exploring nitroarene chemistry. A potentially exciting direction for this field would be to see whether 1,3-2-dioxazolidines can be used as reactants for other useful chemical transformations.

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