

Pyroptosis of fat

DR DIANE DUNCAN discusses the BodyFX treatment and how cell death mechanisms affect clinical results

When we talk about ways to destroy fat, we loosely use the terms necrosis and apoptosis because these are the mechanisms that are most commonly known. Here I'd like to highlight the importance of bearing in mind the particular way in which a cell dies. Consideration of how the cell dies and how this mechanism of death will affect the clinical outcomes is an important and somewhat overlooked premise.

Ways to kill fat

Until recently we've only thought of the two polar opposites of ways to kill fat. Apoptosis is really silent cell death and this is what happens when our cells die in order keep the total population of cells stable. About 100,000 cells per second undergo apoptosis in a human because our cells also divide and undergo mitosis. A balance must be kept. As you know embryos tend to have a tail, and it's apoptosis that makes the cells of that tail disappear without a scar. If we didn't

have programmed cell death we would be massive creatures.

By definition there's no inflammation at all when an apoptotic process occurs.

Necrosis, on the other hand, is severely inflammatory, but it is not the ideal mechanism for fat reduction either. Necrosis causes an instant demise of the affected cell. The cell membrane ruptures, causing the release of lysozymes into the surrounding tissue and the involved cells undergo significant swelling. Another word for the process is "oncosis", as necrosis is more correctly used as identifying the cell when it is dead.

Necrosis and apoptosis

Why is neither necrosis nor apoptosis an ideal mechanism when contouring the face, neck, or body?

In apoptosis there is no inflammation, and in necrosis, the swelling and bruising cause significant down time. Controlled inflammation is desirable, as with time, the body loses its support system for soft tissue (figure 1). A 23 year old

has a thick, sturdy fibrovascular network that holds the fatty layer together and binds it to the underlying fascia and overlying skin. A 44 year old has lost about 50% of this support network, allowing regions of fat to become pendulous and quite saggy. By age 60, about 85% of the fibrous tissue binding fat cells together has eroded away. When we use the term "skin laxity", we are actually talking about a combination of the skin and adipose layer. The loss of attachment to the underlying fascia, plus the loss of a scaffold that is knitting the fatty layer together, cause the flabby character of ageing skin and soft tissue. In many cases, the nature of the soft tissue is actu-

CELL DEATH

Apoptosis is a normal, always ongoing process in humans that programs cells to die in order to offset the number of new cells created by mitosis. Necrosis is immediate, sudden, and very inflammatory, so patients treated with a necrosis inducing regimen—such as injection lipolysis—will see a lot of swelling and bruising. Both necrosis and pyroptotic-like mechanisms are pro-inflammatory. One has poration and one does not. One has cell shrinkage and one is characterized by cell wall rupture.

Apoptosis and necrosis are not the only mechanisms of cell death; others include autophagy, pyroptosis, caspase independent apoptosis, paraptosis, Wallerian degeneration, cornification and anoikis.





ally more important than that of the skin.

Soft Tissue Contouring

The ideal mechanism for soft tissue contouring would be somewhere between apoptosis—with no inflammation, and necrosis' rampant

scar tissue formation.

A desired outcome would be restoration of the youthful fibrovascular support system to the adipose layer. This would require a fractional and somewhat uniform response—layers of tendrils of collagen that are interspersed within the fatty layer. A multilevel response would be needed, and the response would need to be able to be controlled—more fibrous support in some areas, like the jowl or lower abdomen and a bit less in areas that do not tend to be pendulous, like the cheek, or outer thigh. The fibrous support response alone can be generated with a moving external radiofrequency device like Forma or Plus.

Results and temperature

I did a study in which I looked at serial scanning electron microscopy sections of fat, treated over time with Forma at different maximum temperatures: 40, 41, 42, and 43 degrees Celsius. We looked at adipose cells immediately following the eighth weekly treatment, at one month following treatment cessation, and again at three months following treatment cessation. We had two questions: one, could moving RF alone kill fat? And two, does a hotter temperature which may not be well tolerated by the patient create a better tissue response? Figure 2 shows a matrix of SEMs showing tissue response over time at different temperatures. Temporary cell deformation increases with higher temperatures. If you read the thermal literature, fat must get to about 55 degrees Celsius in order to cause necrosis. It is extremely difficult to create that level of heat transcutaneously with external RF alone. Interestingly, at one month and three months, all adipocytes had recov-

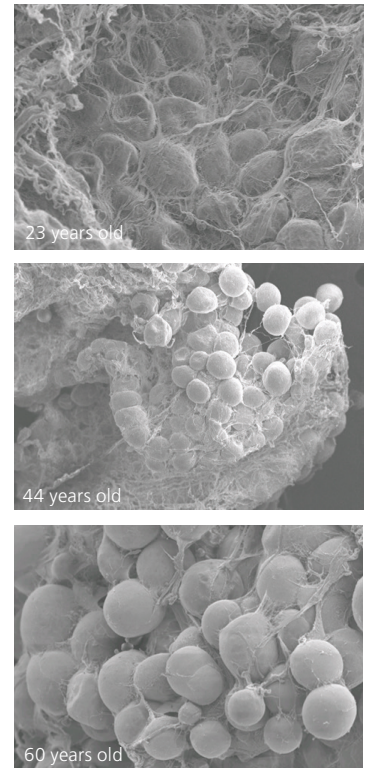


Figure 1: Young adipose tissue has a strong fibrovascular support framework. Middle aged tissue has lost part of the binding and structural tissue. Older tissue has retained very little remaining connective tissue, allowing the soft tissue to appear lax and pendulous

ered, and no fat death was noted with Forma treatments.

Contrary to the belief that bulk heating, especially radio frequency bulk heating, doesn't work, I have proof that it does. However, the level of fibrous ingrowth into the adipose layer did not vary as significantly with temperature level as originally thought. We've been instructed to get the tissue as hot as possible, however even at 40 degrees, there was a significant tissue response in treated areas. Patients tend not to come back to repeat painful experiences. Clinical results are just as good with lower temperatures and longer treatment times.

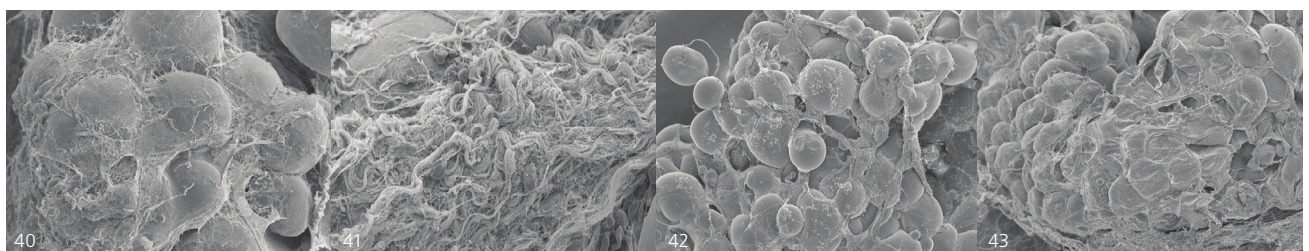


Figure 2: Effect of temperature difference on fibrous tissue response at three months post treatment with moving external RF only. All tissue has fibrous and vascular ingrowth. More cell deformation is present at 43 degrees. If tissue support and molding is the goal, keeping the temperature at 42 degrees or below may be best, as a small amount of fat may die at higher temperatures.

Repeated Measures ANCOVA	Before treatment Mean/ SD	After 1 month Mean/ SD	After 3 months Mean/ SD
US @ 0 degrees	2.63+/- .283	1.74 +/- .202	1.68 +/- .218
US @ 90 degrees	2.83 +/- .160	1.57 +/- .164	1.55 +/- .188
US @ 180 degrees	2.60 +/- .249	1.71 +/- .184	1.76 +/- .204
US @270 degrees	2.82 +/- .237	1.49 +/- .150	1.57 +/- .179
* p<.<.05 The standard for statistical significance			
**p<.<.001 Stronger statistical significance			
The repeated measures analysis (ANCOVA) has been adjusted for age and height			

Figure 3: High resolution ultrasound fat thickness measurements. Multivariate ANCOVA : 53.25% fat thickness reduction at three months post last treatment

Tissue tightening and fat reduction

A device like BodyFX also has moving external radiofrequency. The handpiece is suction-coupled, which means that the target tissue is firmly held so that heat can better get to it. The depth of energy penetration is also controlled this way. However, the secondary electrical impulse is the secret ingredient that makes the fat die.

One of the recent advances in medicine is reversible electroporation, which is used to introduce small molecules into a cell, cause cell fusion, or enable genetic alteration. This causes temporary relaxation of the pores in the cell membrane of target cells, so that medicine or other “genetic directions” can get through. A newer and rapidly growing field, especially in the arena of cancer treatment, is irreversible electroporation. With reversible electroporation, cells can be altered, but none die. With irreversible electroporation, the holes in the pores cannot be reversed, and the cells are programmed to die over time.

The importance of death over time

Most people understand that the inflammatory process in humans takes time. There is about a six-week healing process after surgery and the strength of the repair of a surgical incision gains about 10% per month. This is a process that cannot be hurried. If adipose cells die over time, there is no discomfort, no swelling, no bruising, and no down time. With simple external RF heat, the pattern of fibrous infiltration has barely begun at the end of the eighth treatment, is stronger one month following treatment cessation, and has become quite visible at three months. A similar process is seen with the Body FX; fat takes at least three months to optimally remodel. Fractional cell death is seen; that means not all of the fat cells in a given region die, but a significant number do. Fibrous support is restored, and fat layer thickness is reduced by an average of 40% as measured by high-resolution ultrasound (figure 3).

With the BodyFX, the external RF heat is only the introduction—it sensitises the adipocyte and

lowers the poration threshold. It doesn’t really cause induction of fat death—we treat anywhere between 40°C and 43°C—but it does cause the induction of fibrous response. Different devices that claim fat loss with only moving RF are not very likely to work, given this research. I’ve done a lot of scanning of what happens when tissues are heated using scanning electron microscopy, SEM, which is much easier for people to understand than histology. Adipocytes are notoriously difficult to fix and cut in histologic sections as the cells can easily fracture with processing. The SEM doesn’t have those artefacts. What you see with electron microscopy cannot be manipulated.

Using an SEM I have studied pieces of tissue as a control (figure 4) that have been treated with heat and vacuum alone without the high voltage pulses. These high voltage pulses are the a third step and the patient will feel this as a thump, since a feeling of an electric shock would really be an adverse stimulus. Mechanically the large size of the fat cells is what makes them more susceptible to injury. Their blood supply is poor, so they are

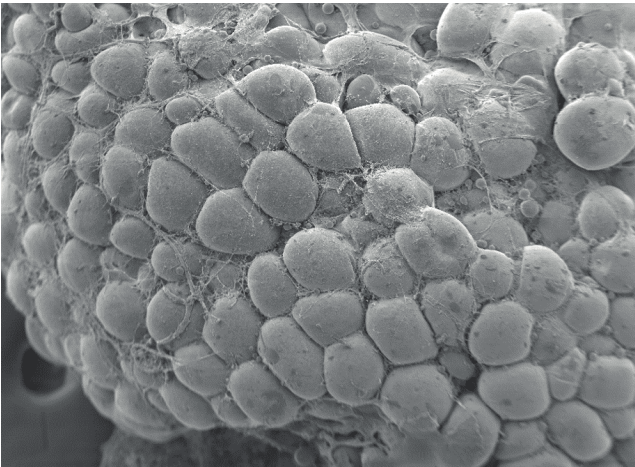


Figure 4: Tissue treated with external RF plus vacuum but no high voltage pulses shows no adipocytolysis at three months. Cells appear similar to tissue treated with external RF alone.

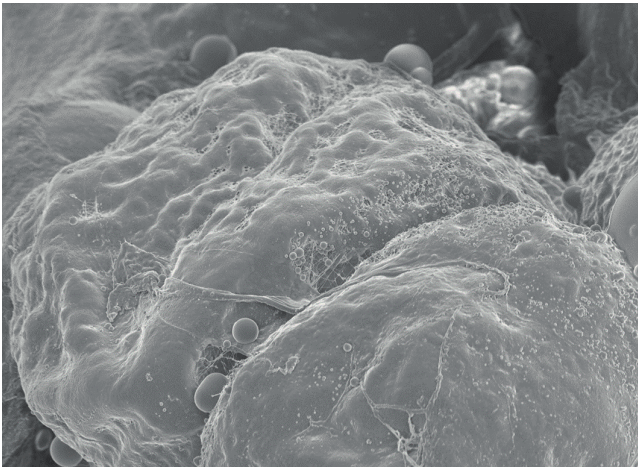
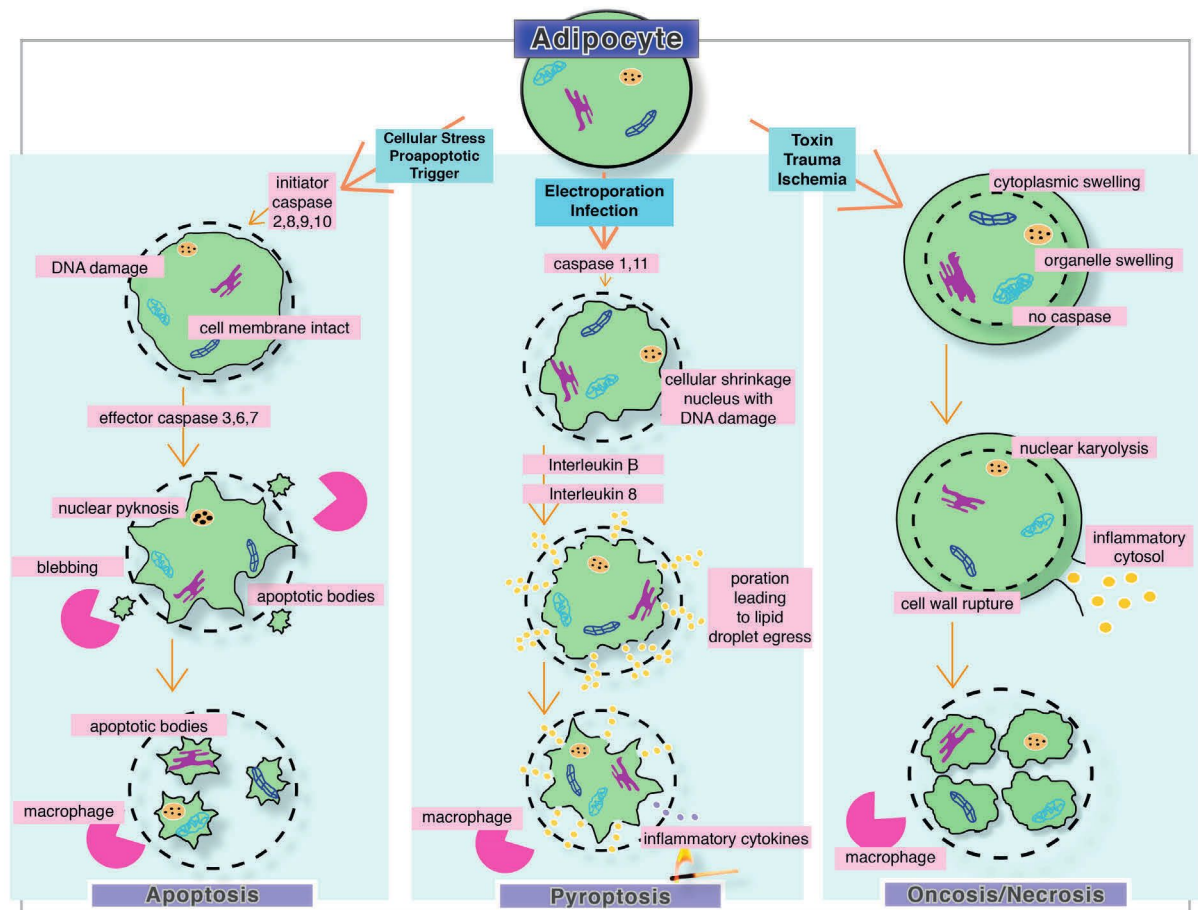


Figure 5: Adipocytes treated with Body FX including high voltage pulses, effect at three months. Note lipid droplet egress continues. Critical volume loss has occurred



How do apoptosis, necrosis and pyroptosis differ?

In looking at the apoptotic process, you can see some cellular budding and eventual separation of these buds (apoptotic bodies). Macrophages will clean up cellular debris. By definition, there is no inflammation. The mechanism is caspase mediated. With necrosis, immediate swelling and bursting of cells causes significant inflammation in the affected area. It is not caspase mediated. Pyroptosis is caspase mediated, which explains why early researchers thought the Body FX mechanism was apoptotic. Also, apoptosis occurs over time, so the slow response with no down time was assumed to be apoptotic. With pyroptosis, poration causes the cell to become "leaky". Cell biology literature notes that when a cell cannot repair the damage to its membrane, it will go on to die.

more easily damaged than other smaller cells.

SEM images (figure 5) show how tissue looks over time after treatment with the BodyFX device with the high voltage pulses. The lipid droplets leak out, or leave the cell through these membrane defects. In looking at thousands of treated cells, we note very few have actual membrane rupture. The membrane has many micro-injuries—and there must be enough, of a degree that cannot be repaired by the cell, in order to cause permanent cell death.

Pyroptosis

Originally discovered by Brad Cookson in 2001 pyroptosis is a pro-inflammatory mechanism. Cookson discovered pyroptosis

while looking at people who had infection with salmonella and shigella and observing that the cell creates little pores in the cell membrane. Some of the cytosol then leaks out, cells shrink and then cytokines signal the cell to die. Pyroptosis is the current best model to help explain what is going on in the Body FX process, but the process is not identical.

In order to see the whole picture I took some control samples of fat tissue injected with saline, but no mechanical or electrical impulses, to illustrate what a normal area of fat cells look like for comparison. The early effect of the BodyFX is totally different than anything that we've seen before. You can see some early cracks in the cell membrane, which is interesting because

you can see the fibrocyte trying to mend that crack so the cell won't die. In breaking down the mechanics of cell death we know that if the fibrocyte can mend that tear then the cell won't die—but that it can't survive with many tears and a lot of lipid droplets that egress, or significant volume loss.

The current gold standard in fat reduction is liposuction. Liposuction removes some, but not all of the fat, so that the tissue that remains behind still acts like "soft" tissue. After liposuction, the remaining tissue can glide over underlying structures, the surface ideally remains smooth, and the fatty layer is reduced. With Body FX, a similar response is seen; fat reduction occurs, but it may not be as significant as with liposuc-

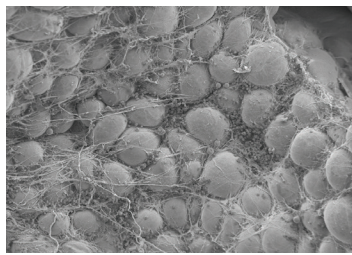


Figure 6: Adipocyte size reduction 6 weeks post cryolipolysis. Average adipocyte size varies from 50- 200 microns, with a mean size of 100 microns. Most adipocytes in this field of treated tissue measure less than 100 microns; all appear viable.

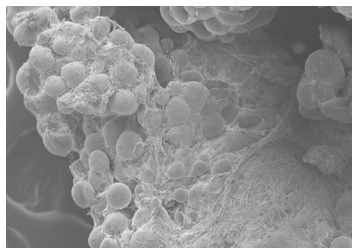


Figure 7: Four months following cryolipolysis, viable fat cells show persistent size reduction in the treatment region, although it is less uniform. 100 microns is the average adipocyte diameter. Note extensive fibrosis in this patient, control untreated tissue appears on the left.

tion. However, there is not a lot of residual scar tissue, and tissue lift can clearly be seen.

Cryolipolysis

As another control study, I looked the mechanism of action of cryolipolysis because I wanted to compare it to that of RF. The officially agreed mechanism of cryolipolysis is apoptosis. However, by comparing the tissues under the SEM it becomes apparent that this cannot be true. The mechanism appears to be mechanical in the early stages. In early SEMs you can see little crimps or folds in the cell wall, most likely due to a combination of the vacuum and the freezing that creates a crystalline structure within the cell. You get some membrane peeling, probably due to the massage performed immediately following treatment, but there's no poration. The lipid droplets don't really come out of pores in cryolipolysis. At four months post-treatment with cryolipolysis, no cell death was seen in these SEM specimens. A uniform reduction of adipocyte size is clear (figure 6). Fibrosis is also evident, which can clinically translate as improvement of tissue pendulosity and a firmer character (figure 7).

These two new mechanisms are

really interesting and we're barely on the verge of understanding them. The radio frequency-induced mechanism is pyroptosis-like. Currently we call it "poroptosis" because the mechanism appears to be irreversible poration that doesn't have any real relationship with the infectious process of Cookson's. The cryolipolysis mechanism of action is clearly not inflammation-free; it's not apoptosis, but it's not necrosis either. We didn't see a single ruptured cell in our SEMs. Cryoptosis has a very different mechanism of action than anything we've seen before.

Age differences

There are age-related differences as well as ethnic variations in tissue type. You can see at age 23 you have a lot more inherent fibrosis or fibre support of the fat tissue, at age 44 there are patches where the support tissue is gone. By age 60 the fat cells are barely held together with little threads of fibrotic tissue. Darker skin type patients tend to have more fibrotic soft tissue, which explains the lesser effect of transcutaneous treatments such as cryolipolysis and radiofrequency at lower settings; the adipose tissue is insulated from both heat and cold by the extra amount of fibrous tissue. By knowing these things, we can optimise the clinical outcome. Fat reduction plus tissue lift, I think, gives the best outcome in most patients.

In looking at before and after images from the BodyFX, you can see some definite tissue lift as well as fat reduction. This is great for older patients who don't have a lot of fibrotic tissue remaining; they get top results because there is a new collagen scaffold that's built up. Paradoxically, with this treatment, middle aged and older patients may see more results than youthful patients with firm tissues.

Conclusion

The ideal mechanism inducing adipose cell death is not necrosis, because there's too much downtime and possible subsequent scarring. Nor is it apoptosis, because if you don't get any inflammation at all you won't get any correction of soft tissue and overlying skin laxity. Poroptosis is fractional which is

ideal, as some, but not all of the fat is affected. There is some inflammation, and I believe this is a good way of inducing fibrous scaffold restoration. However, cryolipolysis is also very interesting because this is also pro-inflammatory. We're just beginning to study both of these mechanisms and clearly more work needs to be done.

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Top: 52 year old before and three months following eight Body FX treatments. Weight gain was one pound. Bottom: 63 year old before and three months following eight weekly treatments. Fat reduction and soft tissue laxity correction has occurred.

