QMP's Plastic Surgery Pulse News

Late to the Party? Pyroptosis Becomes a New Mechanism of Cell Death for Adipocytes

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For many years, medical practitioners have thought that cells die in one of only two ways: Either necrotic cells die violently when their outer membrane ruptures, or cells undergo programmed silent cell death through a mechanism called *apoptosis.* In 2001 Cookson and Brennan[1] published an article in *Trends in Microbiology* describing a new means of cell death that occurs usually within the context of an infectious process called *pyroptosis.*

This method of cell death exists in a continuum between the opposite processes of necrosis and apoptosis, and it exhibits characteristics of both.[2] Pyroptosis is caspase mediated, but its initiator is caspase-1 instead of caspase-2, -8, -9, and -10, which initiate apoptosis. Because the full pyroptotic process takes time, the severe swelling that characterizes tissue response to necrosis is absent. Pyroptotic cell death is not totally silent, however—lysosomal exocytosis is also a marker; therefore varying degrees of inflammation can be seen with this mechanism.

Current Options for Fat Reduction

Why would medical practitioners, especially those with an interest in modifying the subcutaneous soft tissue layer, have any interest in this news? Fine modulation of the soft tissue response, especially with noninvasive fat-reducing devices, has not been possible until recently. Let's look at the current options for fat reduction and some of their limitations.

Liposuction is currently considered the benchmark for focal fat reduction. Dermolipectomy is preferred for patients who have a combination of moderate to severe skin laxity and localized lipodystrophy. Although both techniques are effective, savvy consumers are demanding something more than these old standbys. New energy-based devices lure patients with the prospect of soft tissue and skin tightening with fat suctioning procedures. However, most of the devices that have proven long-term efficacy involve a surgical procedure.[3]

Driven by consumer demand, the development of totally noninvasive devices designed for focal fat reduction has flourished during the past decade.[4] However, most noninvasive body contouring devices offer only a temporary improvement. Those that cause permanent fat reduction include devices using cryolipolysis and those that employ either high-intensity focused ultrasound or cavitational ultrasound.[5] CoolSculpting (Zeltiq, Pleasanton, CA) causes apoptosis of some of the adipocytes in the treatment region. Both Liposonix (Solta Medical, Inc., Hayward, CA) and UltraShape (Syneron, Inc., Irvine, CA) cause necrosis of a contiguous segment of fat in a rectangular treatment region. All three devices use expensive disposables, and the treatment region is dictated by the size and shape of each applicator. Simultaneous soft tissue tightening has not been proven with any of these.

The proliferation of devices using fractional technology has taught us that treatment of only part of the target tissue has a great deal of merit. A noncontiguous tissue response tends to result in a smoother, less operated appearance. If all fat is eradicated in a particular region—usually with a necrotic mechanism of action—the soft tissue response is usually significant to extreme subcutaneous scarring, as seen in the scanning electron micrograph (SEM) images in Fig. 1. In this scenario, very few adipocytes are left in the treatment region. The result? Insufficient *soft* tissue is preserved. Tethered scar tissue formation just under the skin reduces its ability to glide and can cause waves or divots in the skin. Therefore the youthful character of the skin is better preserved by the fractional response of cryolipolysis than by an ultrasound device. A disadvantage of devices that cause necrosis is the effect on the overlying skin. Skin contour irregularity is a well-known risk of traditional liposuction. Many informed patients balk at the idea of trading chubby thighs for bumps and divots. "I'd just be paying to trade one problem for another," noted one of my older, thin-skinned patients during a consultation.

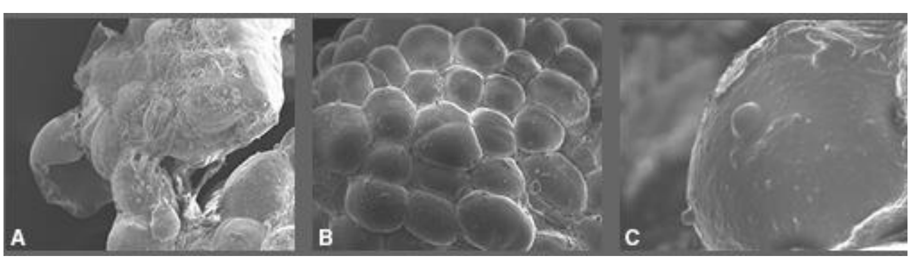

Fig. 1 **A,** SEM of a necrosis response in adipose tissue. The cell membrane has ruptured, and few viable adipocytes remain. **B,** Normal fat is injected with saline as a control. **C,** Close-up of an adipocyte undergoing apoptosis. Blebbing is present in the intact cell membrane.

An advantage of cryolipolysis is the reduction in risk of skin surface irregularity. Its mechanism of action, apoptosis, has the defining characteristic of a lack of inflammation. As more and more patients are treated with this modality, more numerous treatments have been recommended to meet patient expectations.[6] A limitation of this treatment type is that adipocytes are much more sensitive to heat than they are to cold.[7] Although adipocyte death is permanent with these treatments, the lack of inflammation in the hypodermis results in little to no visible soft tissue tightening. In many cases, the generation of a little inflammation within the subcutaneous layer can be a good thing. Many patients lose the support of the fibroseptal network as they age. This typically holds the fat globules together and anchors the skin-fat complex to the underlying fascia. A firm connection of the skin–soft tissue layer to the underlying fascia helps to provide a youthful appearance. As seen in SEM images, flabby tissue significantly lacks fibrocytes within the adipose layer. A young person with a taut physique shows a higher percentage of fibrous tissue than fat tissue (Fig. 2). Fibrous fat tends to have less of a pendulous overhang than puddinglike fat.

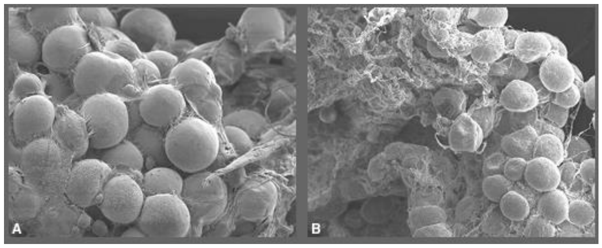

Fig. 2 **A,** SEM of adipose tissue in a 60-year-old woman with type II skin. **B,** SEM of adipose tissue in a 24-year-old woman with type III skin.

Radiofrequency Energy and Pyroptosis

An investigation for a device that causes an intermediate reaction—a fractional response, permanent adipocyte demise, and a mild to moderate inflammatory response in the treatment region—revealed a common device type based on radiofrequency (RF) energy. In patients with mild to moderate skin laxity, RF has been shown to be the optimal energy source for creating soft tissue and overlying skin tightening.[8]

How does RF energy affect soft tissue? The noninvasive model of the BodyFX device (Invasix, Yokneam, Israel) involves constant suctioning and a combination of thermal modulation of the soft tissue with ultrashort oscillating waves of high-amplitude pulses to create a tissue response known as *electroporation.* Reversible electroporation is used to drive molecules into otherwise impermeable cell membranes.[9] Irreversible electroporation (IRE) is a rapidly developing field, especially in the arena of cancer therapy.[10] This process occurs with the Invasix device. It involves the development of many tiny pores or openings in the affected cell wall in a manner very similar to that of a pyroptotic response. Extracellular calcium flows into these defects, eliminating the efficacy of the ion pump system. A similar process is seen in the response of cells to salmonella, shigella, and anthrax infection. Lysosomal exocytosis then occurs. A hallmark of the pyroptotic response is a clear loss of cell volume after the initial swelling. Whereas a necrotic cell membrane undergoes lysis immediately, a pyroptotic cell shows many tiny tears and leaks. Intracellular lipids egress, and the cell signals to itself to die over time.

Patient Results on Scanning Electron Micrographs

SEMs were obtained from serial biopsy samples of one patient treated with a BodyFX device (Fig. 3). The direct effect on adipocytes was evaluated over a period of 8 weeks. Fig. 3, *A,* shows a normal, untreated adipocyte. The depression on the top of the cell was created by a nesting adipocyte. The cell surface had a thin, fibrous coating. Adipocytes were held together by a filmy network of fibrocytes. Fig. 3, *B,* shows the effects of electroporation on an adipocyte 2 weeks after two treatments with an RF-based noninvasive device. Small cracks were evident in the cell membrane. These appeared to be more mechanical in nature than the more round and uniform poration caused by infectious agents in classic pyroptosis. Fig. 3, *C,* shows the adipocytes at 4 weeks, immediately after four treatments of RF. The cell wall revealed a dense, fairly uniform poration effect. Small bubbles under the membrane surface are lipid droplets that will egress through the cracks in the cell wall. Fig. 3, *D,* shows a fractional response 8 weeks after the final (eighth) RF treatment. Not all cells were affected. Normal adipocytes (*left* and *upper right*) were adjacent to cells that had lost significant amounts of cytosol. Damaged cells undergo DNA fragmentation in the nuclei; these cells send signals to themselves to die. Fig. 3, *E,* shows lipid droplets extruding through the pores of the affected adipocyte. This reaction, seen at 8 weeks, is one of the hallmarks of the pyroptotic process. With apoptosis, the cell membrane remains intact. The necrotic process is marked by the early lysis of the cell wall, with sudden release of the cytosol and a strong inflammatory response. In pyroptosis, poration is an early response, with gradual development of fractional cell death.

Fig. 3 **A,** A normal untreated adipocyte. The depression on the top of the cell was created by a nesting adipocyte. The cell surface had a thin fibrous coating. Adipocytes were held together by a filmy network of fibrocytes. **B,** Effects of electroporation on an adipocyte are shown 2 weeks after two treatments with an RF-based noninvasive device. Small cracks were apparent in the cell membrane. **C,** At 4 weeks, immediately after four treatments of RF, the cell wall had a dense, fairly uniform poration effect. Small bubbles under the membrane surface were lipid droplets that will egress through the cracks in the cell wall.

The clinical advantage seen with this poration-type soft tissue response is definite. Fractional adipocytolysis preserves the character of the soft tissue and prevents skin contour irregularities that can be seen with a necrotic response. The generation of a mild to moderate inflammatory response can enhance a lax fibroseptal network. The best responders to this RF-based tissue treatment type are older patients with little fibrous content in their fatty layer. A thick fibroseptal network can act as insulation, creating a protective covering for the adipocytes, which are then less affected by the heat and electroporation effect.

Ethnic and age-related differences in soft tissue types can explain the variation in response to treatment with many energy-based noninvasive body contouring devices. Chan[11] noted that Asian patients responded less well than white patients to multiple treatments of cryolipolysis. The ratio of fibrous tissue to adipocytes can directly affect the response of soft tissue to thermal and acoustic treatments.

Conclusion

Although early evidence shows that irreversible electroporation causes a pyroptotic-type response in treated adipocytes, the mechanism of action seen with SEM varies somewhat from the process seen with infection-caused pyroptosis. The recent emergence of similar but distinct mechanisms of cell death such as anoikis, pyronecrosis, paraptosis, and entosis shows that there are probably many undiscovered cell death pathways, each with unique characteristics.[12] More study is needed to better define the step-by-step mechanism of action of irreversible electroporation on adipocytes. As the nuances of response to increases and decreases in power, duration, optimal treatment temperature, wave oscillation patterns, and subject tissue type are studied, we will be better able to predict and control treatment outcomes.

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