# Piezo-therapy in cancer: Current research and perspectives

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## Abstract

In this article, we introduce the innovative nanotechnological approach of remote electric stimulation mediated by ultrasound-sensitive piezoelectric nanoparticles, especially focusing on its exploitation in the nanomedicine field for the "wireless" anticancer electric treatment. The nanoparticle functionalization with specific ligands allows the targeting, the imaging, and ultimately the treatment of cancer cells. Piezoelectric stimulation can be performed in remote modality with ultrasound waves by virtue of the direct piezoelectric effect. Chronic piezo-stimulation is able to remarkably decrease cancer cell growth by inducing the cell cycle arrest in G0/G1phase and by affecting the cytoskeleton organization during cell division. The reported results indicate an impressive potential impact of this nanotechnological approach that will be further tested in future works in synergic combination with chemotherapy treatments. 

**Keywords:** Piezoelectric nanoparticles, cancer therapy, chemotherapy, electric stimulation.

An innovative and promising anticancer approach able to inhibit cancer cell proliferation and to enhance drug sensitivity is represented by low-intensity electric stimulation. Physical stimuli of low-intensity alternated current (AC) induce antiproliferative effects on different types of human and rodent cancer cells (*e.g.*, Patricia C, U-118, U-87, H-1299, MDA231, PC3, B16F1, F-98, C-6, RG2, and CT-26) by affecting the $K^+$ homeostasis and the organization of cytoskeleton components (*i.e.*, tubulin of the mitotic spindle) involved during mitosis [**1, 2**]. Moreover, an increased sensitivity of glioblastoma cells to the treatment with temozolomide (TMZ) drug, a chemotherapy commonly used in clinics, was observed when combining the AC stimulation with TMZ therapy [**3-5**]. The mechanism of AC-induced decrease of TMZ resistance seems to be mediated by an impairment of the P-glycoprotein (P-gp), the overexpression of which is associated to multidrug resistance.

Despite low-intensity AC displays promising anticancer effects against different types of cancer cells, this approach is known to affect the proliferation of non-malignant cells too [**1**]. In this context, our research aims to develop innovative nanotechnological approaches for the remote delivery of electrical stimuli to cancer cells.

Piezoelectric nanotransducers are smart nanomaterials able to efficiently convert a mechanical energy into electric one thanks to the direct piezoelectric effect. As a source of mechanical energy, ultrasounds (US) resulted to be optimal candidates for safely conveying mechanical waves and for remotely activating the nanostructures [**6**]. Following this approach, piezoelectric nanotransducers have been exploited by our group (**Fig. 1**) as electric nanostimulators to wirelessly excite different types of cells, such as, for example, neurons [**7-9**], osteoblasts [**10**], and cancer cells [**11**].

Specifically, due to their great biocompatibility levels and excellent piezoelectric coefficients, piezoelectric boron nitride nanotubes (BNNTs) and barium titanate nanoparticles (BTNPs) have been extensively investigated [**12**].

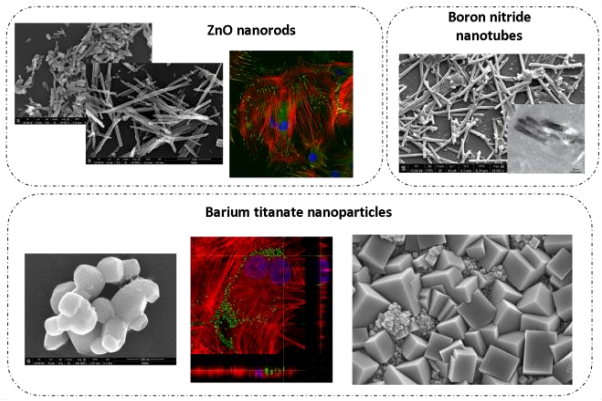


**Fig. 1.** Different examples of piezoelectric nanoparticles and of their interaction with cells. Reproduced with permission from [12]; Copyright 2017 Elsevier.

Particular attention has been dedicated to BTNPs because the synthesis of this nanomaterial is well established and large quantities of BTNPs can be easily obtained with great purity levels. Mathematical models of the electro-elastic behavior of BTNPs indicated as a single nanoparticle of 300 nm in diameter is able to evoke an output voltage ($\varphi$ ~ 0.5 mV) when mechanically activated by US with an intensity of $I_{US}$ ~ 1 W/cm$^2$ [**8**]. Moreover, protocols of chronic US impulses have been developed in order to avoid temperature increases and non-specific cell activations; thanks to this approach, mechanical waves remotely activate nanoparticles without affecting cell behavior [**9, 11**].

Nanotechnology offers the possibility to target nanomaterials to specific cell types by surface modification with specific ligands. As an example, BTNPs of about 60-100 nm in diameter were silanized and covalently conjugated to secondary antibodies (Ab) by Hsieh *et al.* [**13**]. The functionalized BTNPs were shown to specifically bind to anti-human leukocyte antigen labeled HeLa cells.

With a similar approach, our group functionalized BTNPs with anti-HER2 antibody (AbBTNPs) to specifically target HER2 positive cancer cells [**11**]. In this work, US-driven chronic piezo-stimulation of AbBTNPs-incubated SK-BR-3 breast cancer cells induced significant anti-proliferative effects. The piezoelectric stimulation arrested SK-BR-3 cell cycle in G0/G1 phases by interfering with $Ca^{2+}$ homeostasis and upregulating the expression of the gene encoding for Kir3.2 inward rectifier $K^+$ channels. In addition, the organization of cytoskeleton elements mediating cell mitosis was affected: several abnormal mitotic conformations (*i.e.*, tripolar mitosis, multipolar / monopolar spindle and rosette) were observed in response to the chronic piezoelectric stimulation.

Altogether, the presented works highlight the scientific and technologic relevance of this stimulation approach in the field of nano-oncology. The US-driven nanoparticle-assisted piezoelectric treatment acts as "wireless" anticancer therapy by remotely delivering anti-proliferative stimuli to cancer cells (**Fig. 2**). In principle, this platform would allow inhibiting cancer cell proliferation without affecting functions and viability of healthy tissues. The nanosize of the piezoelectric nanostimulators would also allow reaching cancers that are difficult to target and counteract (*e.g.*, brain cancers). For all these reasons, the *in vivo* testing of piezoelectric nanostimulators has become matter of great importance for the development of a safe, efficient, and versatile anti-cancer therapy. The combination of the piezoelectric stimulation with different chemotherapy treatments will be investigated in the close future with the aim to enhance the possibilities of therapeutic success.


**Fig. 2**. Pictorial representation of piezoelectric stimulation of cancer cells mediated by barium titanate nanoparticles (BTNPs).

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