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Research Paper

# AFM-Based Study of Fullerenol ($C_{60}(OH)_{24}$)-Induced Changes of Elasticity in Living SMCC-7721 Cells

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ABSTRACT

In this study, the alterations of the morphology and biomechanical properties of living SMCC-7721 cancer cells treated with fullerenol ($C_{60}(OH)_{24}$) for 24, 48, and 72 h were investigated using an atomic force microscope (AFM). Comparative analyses show that the elastic moduli of the SMCC-7721 cells exposed to fullerenol decrease significantly with the increase of the treatment periods. Furthermore, in different phases of the treatment, a global decrease in elasticity is accompanied by cellular morphological changes, and the time-dependent effect of the fullerenol can be observed using AFM and optical microscope. In addition, as the treatment duration increases, the indentation force and depth penetrated into the cell membrane by the AFM tip are in a declining trend. The reduction in the stiffness of the cells exposed to fullerenol could be associated with the disruption of the cellular cytoskeleton network. The investigation indicates that the elastic modulus of single living cells can be a useful biomarker to evaluate the effects of fullerenol or other anticancer agents on the cells and reveal instructive information for cellular dynamic behaviors.



## 1. Introduction

The fullerene family possesses unique physical and chemical properties for potential applications in biomedicine such as cancer diagnosis and therapy (Chen et al., 2010; Chen et al., 2012; Partha and Conyers, 2009). Native fullerenes ($C_{60}$) lacking solubility result in aggregation in aqueous solutions and have high toxicity (Chen et al., 2010; Nielsen et al., 2008; Partha and Conyers, 2009; Shimizu et al., 2013; Sayes et al., 2004), which hinders their applications in biomedicine, while water-soluble fullerenes, as a class of fullerene derivatives, can be synthesized by the addition of hydroxyl groups onto fullerene molecules and are termed as fullerenol ($C_{60}(OH)_n$). Consequently, some of them improve the solubility of fullerenes and increase their use in biomedicine or pharmaceutical applications (Jensen et al., 1996; Rade et al., 2008). Recently, some studies have reported that the effects of carbon-based nanomaterials on the cytoskeletal structure (Tian et al., 2006; Walker et al., 2009; Dong et al., 2013) and the dynamical changes in the cytoskeleton will affect the cell behaviors, including migration, differentiation, and apoptosis or shape maintenance. Researchers have proposed a number

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of models to study and describe the apoptosis process. In different apoptosis stages, cell shrinkage, swelling, and rounding can be observed in many types of cells (Imajoh et al., 2004; Desjardins and MacManus, 1995; Saraste and Pulkki, 2000). Furthermore, increasing evidence shows that the disruption of the actin cytoskeleton may be an initiating apoptotic event. Among the literatures, Zhou et al. (2006) have suggested that the disruption of the actin cytoskeleton can induce apoptosis. Moreover, Johnson-Lyles et al. (2010) exposed the renal proximal tubule cells to fullerenol and found that the fullerenol cytotoxicity-induced apoptosis is associated with cytoskeleton disruption. In addition, the antiproliferative effect and specific photophysical properties of fullerenol make it a potential antitumor or anticancer agent (Foleya et al., 2001; Lu et al., 1998). More importantly, the identification of cancer cells affected by fullerenols could enhance the understanding of the role and influence of fullerenols, and it could reveal the potential of the cancer progression amelioration by combining nanoscience and biomechanics.

Nanobiomechanics has been demonstrated to be distinctly useful to study cancer development, human disease, and mechanisms of drug actions (Lee and Lim, 2007; Maciaszek and Lykotrafitis, 2011; Rotsch et al., 1997). The investigation of the changes of elastic stiffness in cancer progression is helpful to understand the individual differences between normal cells and cancer cells (Plodinec et al., 2012). A number of studies show that fullerenols can inhibit cancer cells or tumor proliferation and induce apoptosis in exposed cells (Bosi et al., 2003; Chen et al., 2012; Lu et al., 1998). They could inhibit the synthesis of microtubules and disrupt actin filaments (Johnson-Lyles et al., 2010; Mrdanović et al., 2009). Both of them not only serve as structural elements in the cytoskeleton of the cells (Etienne-Manneville, 2004; Fuchs and Karakesisoglou, 2001) but are also able to regulate the mechanical stability of living cells (Unterberger et al., 2013). The alterations of cellular elastic stiffness directly reflect the changes of the cytoskeletons and affect the cellular processes (Aryaei and Jayasuriya, 2013; Mason et al., 2012; Nikolaev et al., 2014). The experimental study of cellular elastic stiffness on micro- and nanoscales could provide instructive information on cancer progression. The mechanical measurement of individual living cells by means of the atomic force microscope (AFM) indentation is accurate (Yoo et al., 2014; Zhu et al., 2011). Since the AFM was invented in 1986 (Binnig et al., 1986), it has been rapidly developed into a powerful tool for nanoscale characterization and modification because of the high sensitivity for the detection of cantilever deformation and realized high-precision controllability on the nanoscale. Moreover, it provides a promising way for the study of living cells in vitro, as three-dimensional (3D) imaging and real-time force measurements of biological cells can be achieved by AFM in physiological environments. For instance, it can be seen, from the indentation force–displacement curves of the cantilever deformation versus the displacement of the piezo scanner, that the elastic moduli of some cancer cells are significantly different from normal cells (Fuhrmann et al., 2011; Lekka et al., 1999; Plodinec et al., 2012). Thus, the elastic modulus of single living cells can be regarded as a biomarker for the metastasis or spread potential of some cancer cells or tumor cells (Cross et al., 2007; Jonietz, 2012; Kumar and Weaver, 2009; Lautenschläger et al., 2009; Li et al., 2008; Xu et al., 2012) and for drug efficacy testing.

Although some efforts have been made to study living cancer cells by AFM in recent years, there is still a lack of knowledge or evidence that clearly confirms the relationships between the biomechanical properties of living cells and their dynamic cellular processes, and there is also no reference that shows the effects of fullerenol ($C_{60}(OH)_{24}$) on the biomechanical properties of living cancer cells. According to the World Health Organization (WHO) investigation in 2012, liver cancer is the second most common cause of cancer deaths (0.8 million, 9.1%) (http://globocan.iarc.fr/Default.aspx) (GLOBOCAN, 2012). In this work, human hepatocellular carcinoma (SMCC-7721) was selected as the research object. An AFM was employed to examine the changes of elastic moduli on the SMCC-7721 cells and the cells were treated with fullerenol ($C_{60}(OH)_{24}$) for 24, 48, and 72 h. The morphological and biomechanical cellular changes caused by fullerenol were investigated using AFM topography and nanoindentation. The investigation suggests that the measurements of the biomechanical properties of single living SMCC-7721 cells treated with fullerenol could be used to evaluate the effect of fullerenol or other anticancer agents on the cells and can represent a crucial part of the potential cancer progression.

## 2. Materials and methods

### 2.1. Cell culture

Human hepatocellular carcinoma (SMCC-7721) cells were grown in Roswell Park Memorial Institute (RPMI)-1640 media with 10% of fetal bovine serum (FBS) and antibiotics (penicillin–streptomycin solution). The cells were maintained in a humidified incubator with 5% of $CO_2$ at a temperature of 37 °C. Flasks containing cells were treated with trypsin. The cells were dispersed and plated on glass coverslips (18 × 18 mm) at a density of $1.0 \times 10^5$ cells/ml (1 ml per coverslip) in 38-mm plastic petri culture dishes and incubated for 24 h at 37 °C.

### 2.2. Preparation of fullerenol treatment solution

The commercial water-soluble fullerenol powder with the general formula $C_{60}(OH)_{24}$ was dissolved in deionized water at a concentration of 2.7 μM/ml, and it was then diluted with RPMI-1640 media with 10% of FBS to 0.53 μM/ml, which was used for the fullerenol treatment solution stored at 4 °C.

### 2.3. Sample preparation

SMCC-7721 cells were dispersed and plated on glass coverslips. After 24 h, they were washed with phosphate buffered saline (PBS) to remove unbound and dead cells, and then continuously incubated with the fullerenol treatment solution at 37 °C in a humidified incubator with 5% of $CO_2$ for 24, 48, and 72 h. To each of the plastic petri culture dishes, 2 ml of the fullerenol treatment solution was added. After incubation, the exposed cells were washed with PBS and measured