Cellular and transcriptomic analysis of human mesenchymal stem cell response to plasma-activated hydroxyapatite coating

Fei Tan, Feidhlim O’Neill, Mariam Naciri, Denis Dowling, Mohamed Al-Rubea

School of Chemical and Bioprocess Engineering, and Conway Institute of Biomolecular and Biomedical Research, University College Dublin, Belfield, Dublin 4, Ireland

School of Medicine and Medical Science, University College Dublin, Belfield, Dublin 4, Ireland

School of Electrical, Electronic and Mechanical Engineering, University College Dublin, Belfield, Dublin 4, Ireland

Abstract

Atmospheric pressure plasma has recently emerged as a technique with a promising future in the medical field. In this work we used the technique as a post-deposition modification process as a means to activate hydroxyapatite (HA) coatings. Contact angle goniometry, optical profilometry, scanning electron microscopy morphology imaging and X-ray photoelectron spectroscopy analysis demonstrate that surface wettability is improved after treatment, without inducing any concomitant damage to the coating. The protein adsorption pattern has been found to be preferable for MSC, and this may result in greater cell attachment and adhesion to plasma-activated HA than to untreated samples. Cell cycle distribution analysis using flow cytometry reveals a faster transition from G1 to S phase, thus leading to a faster cell proliferation rate on plasma-activated HA. This indicates that the improvement in surface wettability independently enhances cell attachment and cell proliferation, which is possibly mediated by FAK phosphorylation. Pathway-specific polymerase chain reaction arrays revealed that wettability has a substantial influence on gene expression during osteogenic differentiation of human MSC. Plasma-activated HA tends to enhance this process by systemically deregulating multiple genes. In addition, the majority of these deregulated genes had been appropriately translated, as confirmed by ELISA protein quantification. Lastly, alizarin red staining showed that plasma-activated HA is capable of improving mineralization for up to 3 weeks of in vitro culture. It was concluded from this study that atmospheric pressure plasma is a potent tool for modifying the biological function of a material without causing thermal damage, such that adhesion molecules and drugs might be deposited on the original coating to improve performance.

1. Introduction

The term plasma is used to describe a collection of charged particles (an ionized gas) in physicochemical science and also the liquid component of blood in medicine. Both plasma types share a cardinal feature: both are macroscopically neutral and microscopically ionized active media [1]. This article focuses on the type of plasma which is generated by applying energy to a gas resulting in a mixture of ions, electrons and neutral species. A plasma can be classified as thermal or non-thermal, depending on its method of creation. Thermal plasma processes have a long history of industrial/physiological tissues [6].

The medical applications of non-thermal plasmas fall into two major types: direct and indirect plasma treatment, differentiated by the amount of charged species applied to the surface of the living tissue. Direct plasma treatment gives off ozone, NO and OH radicals to the surface, with some passing through the living tissue. It is therefore mainly applied in skin sterilization, blood coagulation [7], and assisting wound healing and tissue regeneration [8]. On the other hand, indirect plasma treatment, represented by atmospheric pressure plasma jets (APPJ) [9], delivers reactive species generated between two electrodes to the area of application in the form of a gas flow. Apart from the recently discovered effects of indirect plasma treatment on cell apoptosis [10], necrosis [11] and detachment [12], very little is known regarding its influence on osteogenic lineage cells or bone tissue, let alone its application in surgical repair of hard tissue. In this study, rather than applying APPJ straight to cells, we used an HA coating, which is one of the most commonly used orthopedic biomaterials, as a medium to transfer the effect of APPJ to bone-forming cells. This is possible due to its finely tunable effects and selective influence on pathological/physiological tissues [6].