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Cell biological effects of mechanical stimulations generated by focused extracorporeal shock wave applications on cultured human bone marrow stromal cells

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Abstract Human bone marrow stromal cells (hBMSCs) bear tremendous clinical potential due to their immunomodulatory properties in transplantation settings and their contribution to tissue regeneration. In fact, they are among the most promising types of stem-like cells for therapeutic applications and are the subject of intense research. However, the clinical use of hBMSCs has been confounded by limitations in their availability; they are scarce cells cumbersome to isolate and purify. Additionally, they are difficult to target to the site of injury in regeneration experiments. In order to combat these limitations, focused extracorporeal shock waves (fESW, 0.2/0.3 mJ * mm^{-2}) were applied to purified, cultured hBMSCs. fESW (0.2 mJ * mm^{-2}) stimulations were found to increase hBMSCs' growth rate ($p < 0.05$), proliferation ($p < 0.05$), migration, cell tracking and wound healing ($p < 0.05$, respectively), as well as to reduce the rate of apoptosis activation ($p < 0.05$). The increase in hBMSC migration behavior was found to be mediated by active remodeling of the actin cytoskeleton as indicated by increased directed stress fiber formations ($p < 0.05$). Furthermore,

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hBMSCs maintain their differentiation potentials after fESW treatment, whereas 0.2 mJ * mm^{-2} is the most effective application. In conclusion, our results establish first-timely that hBMSCs' behavior can be modified and optimized in response to defined mechanical stimulation. These findings appear particularly promising as they suggest that mechanical stress preconditions hBMSCs for improved therapeutic performance without genetic manipulations and that mechanically preconditioned hBMSCs will be advantageous for hBMSC-based tissue regeneration. Therefore, this approach opens the door for exploiting the full potential of these cells in regenerative medicine.

Introduction

Human bone marrow stromal cells (hBMSCs) are characterized by their capability to differentiate into various mesenchymal tissues, including adipocytes, chondrocytes and osteocytes (Ciapetti et al., 2006; Kuznetsov et al., 1997a; Meinel et al., 2004; Pittenger et al., 1999; Tondreau et al., 2004). hBMSCs are a promising tool for the field of regenerative medicine for several reasons; they can be cultured ex vivo, have endogenous activation potential (Jiang et al., 2002; Pittenger et al., 1999), can be systemically delivered or undergo allogeneic transplantation (Krause et al., 2007; Wolf et al., 2009) and have a high self-renewal potential (English et al., 2010; Kolf et al., 2007; Nombela-Arrieta et al., 2011; Song et al., 2006; Zaragosi et al., 2006). Their immunosuppressive properties make them a promising tool to counter complications arising from graft versus host rejections in transplantation settings of cells other than hBMSCs (English et al., 2010; Prockop, 2009). It should be noted that the transplantations of BMSCs have not been highly successful, yet, in order to cure disease tissue, as BMSCs disappear within a short period of time after transplantation. However, a myriad of research is still geared towards utilizing their differentiation potential for tissue engineering approaches, particularly for cartilage and bone regeneration (Nombela-Arrieta et al., 2011) indicating the unraveled potential of BMSCs in tissue regeneration.

How hBMSCs exert their beneficial effects in tissue regeneration is, however, less clear. It was first hypothesized that BMSCs engraft directly into the degenerative tissue and differentiate into the respective cell type (Mahmood et al., 2003; Murphy et al., 2003). However, there is accumulating evidence in favor of a second hypothesis postulating that BMSCs predominantly contribute to tissue regeneration by their capability to secrete a variety of trophic factors contributing to paracrine effects in tissue repair (Gnecchi et al., 2005; Haynesworth et al., 1996; Ladage et al., 2007; Mirotsou et al., 2007) rather than tissue-specific differentiation (Prockop, 2009). Regardless of how BMSCs unfold their remarkable healing properties, it has been demonstrated in different studies that only a low percentage of transplanted hBMSCs reaches the targeted disease area (Hofmann et al., 2005) and that after a few days, only a small amount (2–6%) persists in the affected tissue (Hofmann et al., 2005; Kolf et al., 2007). In addition to the limitation of the presence of BMSCs at the site of injury, certain proportions of these cells need to display proliferative and apoptotic, as well as migratory, behaviors in order to mediate tissue repair (Li and Jiang, 2011; Schmidt et al., 2006). Thus, the therapeutic success of using hBMSCs in tissue regeneration has been limited and falls far behind their potential.

In order to increase the efficiency of targeting hBMSCs to the disease area and to promote their beneficial cellular response at those desired targets for therapeutic use, novel strategies avoiding genetic manipulation are needed (Karp and Leng Teo, 2009; Peterson et al., 2011; Schumann et al., 2006; Yan et al., 2011) to precondition BMSCs (Ghanem et al., 2009). Extracorporeal shock waves (ESW) are transient pressure fluctuations that propagate 3-dimensionally and that are widely applied in the context of therapeutic mechanotransduction with a high success of increased tissue regeneration (Nishida et al., 2004; van der Jagt et al., 2011, 2013). Two major types of ESW are used in medical therapies, focused and radial ESW, whereas the former ESW type reflects high-peak pressure amplitudes and the majority of energy flux is concentrated on a small focus (Chang et al., 2012). These data show that focused ESW (fESW) types might be a suitable tool for preconditioning hBMSCs in order to improve their therapeutic potentials.

It is known that the expression of genes involved in differentiation pathways of hBMSCs can be influenced by mechanical stimuli (Friedl et al., 2007). Different groups have shown that ESW offer great beneficial potential in the treatment of patients suffering from different conditions, including injured bone, cartilage and cardiac tissue (Nishida et al., 2004; Wang et al., 2003). Unfortunately, the mechanistic foundation for these improvements has not been addressed in these studies and the identity of cellular contributors has not been confirmed. In this context, no knowledge exists concerning the potential effects of ESW application on cell biological properties of hBMSCs, such as proliferation and migration, that are crucial for successful medical BMSC-based therapies (Li and Jiang, 2011). Additionally, it remains unclear whether hBMSCs maintain their full differentiation potentials after treatment with mechanical stimuli.

To address the unresolved, but highly significant question of whether hBMSCs' behavior can be manipulated by ESW application and whether those manipulations depend on energy and/or timing of ESW application as well as whether hBMSCs maintain their full differentiation potentials after ESW treatment, the present study hypothesized (I) that ESW applications induce cell biological effects on purified cultured hBMSCs that prove beneficial for tissue regeneration, (II) that the cell biological processes are dependent on the dose of ESW application and (III) that hBMSCs maintain their full differentiation potentials after ESW applications. The results clearly demonstrate that ESW promote biological processes in hBMSCs, including increased proliferation, survival and migration, which are described to prove beneficial for tissue regeneration. Additionally, ESW do not disturb hBMSCs' differentiation potentials. Therefore, the presented observations demonstrate that ESW stimulations of a defined nature are a dynamic approach to manipulate hBMSC behavior in vitro in order to exploit their full regenerative capacity in vivo. Furthermore,