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Cell-induced response by tetracyclines on human bone marrow colonized hydroxyapatite and Bonelike®

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Abstract

Semi-synthetic tetracyclines are commonly used antibiotics that also seem to play an important role in the modulation of the immuno-inflammatory imbalance, verified in several bone diseases. The association of a therapeutic agent (that prevents bacterial infection and induces tissue formation) to a biomaterial aiming to repair/regenerate bone defects could contribute to a more predictable clinical outcome. The present study intends to evaluate the proliferation and functional activity of osteoblast-induced human bone marrow cells, cultured on the surface of hydroxyapatite (HA) and Bonelike®, in the presence of therapeutic concentrations of doxycycline and minocycline. First passage bone marrow cells were cultured for 35 days on the surface of HA and Bonelike® discs, in the absence or presence of 1 μg ml^{-1} doxycycline and minocycline. Cultures performed in standard tissue culture plates were used as control.

Doxycycline or minocycline induced cell proliferation and increased the extent of matrix mineralization in osteoblastic cell cultures established in the three substrates. Also, an improved biological behavior was verified in seeded Bonelike® compared with HA. The results suggest that the local delivery of tetracyclines might associate the antimicrobial activity in implant-related bone infection with an eventual induction of osteoblastic proliferation and maintenance of the characteristic biological activity of these cells.
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Keywords: Tetracyclines; Osteoblasts; Hydroxyapatite; Bonelike

1. Introduction

Tetracyclines are commonly used bacteriostatic antibiotics active against a wide range of both aerobic and anaerobic Gram-positive bacteria. Their antimicrobial activity is due to the inhibition of bacterial protein synthesis, by binding to the 30*S* ribosome subunit, blocking the bond to the tRNA, on the mRNA–ribosome complex [1].

In the last years, several observations have testified the therapeutic effectiveness of tetracycline (as well as its semi-synthetic derivatives, minocycline and doxycycline) in the modulation of the immuno-inflammatory imbalance verified in several animal and human bone diseases [2–4]. Different mechanisms, distinct from the antimicrobial action, have been proposed to justify the pro-anabolic activity of these pharmacological agents regarding bone metabolism. These include enhancing bone formation, decreasing connective tissue breakdown and diminishing bone resorption [5–10]. Clinical application of these agents targeting bone might also be helped by their cation quelation activity and consequent avidity for mineralized tissue [11].

Ceramic-based biomaterials are currently used in bone tissue repair strategies because of their adequate mechanical properties and chemical composition similar to those of the bone tissue. One of the biomaterials, hydroxyapatite (HA), has generated a great deal of interest in the last years

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[12]. This synthetic bone graft substitute, although lacking osteogenic properties that can only be found in autologous grafts, still offers advantages that include a reduced local tissue morbidity, the absence of complications at the donor site and unlimited material availability [13]. This biomaterial, being similar to the mineral component of natural bone, revealed good osteoconductivity and bone bonding ability [14]. However, HA presents low load-bearing capacity [15], and the introduction of phosphate-based glasses as a sintering aid is known to reinforce HA mechanically [16]. Glass-reinforced HA with bioactive properties has been applied with success in medical and dental clinical applications aiming to regenerate the bone tissue [17,18]. Bonelike® is a registered and patented glass-reinforced HA with improved mechanical properties and enhanced bioactivity that result from the addition of $CaO–P_2O_5$-based glasses during the liquid phase sintering process of HA, leading to several ionic substitutions in the lattice that are responsible for the reduction of porosity and grain size [19,20]. Recently, it has been successfully applied in regenerative procedures aiming to restore bone structure and function in oral, maxillofacial and orthopedic procedures [21,22].

Despite the wide application of synthetic biomaterials in the repair/regeneration of bone tissue, several clinical complications have been established and have proved difficult to remedy. Among them are osteomyelitis, septic arthritis and prosthetic joint infection, which are caused specially by Gram-positive organisms and are known to contribute to a heavy clinical and economic burden [23]. Treatment is often complicated at sites of reduced vascularization, requiring prolonged antimicrobial use, usually associated with surgical drainage or debridement [24]. In this way, the selection of the most effective therapeutic approach, based on several parameters that include the specificity of the pathogenic agents, their sensitivity profile, pharmacokinetics of the drug, local vascular supply, and the presence or absence of a biomaterial and individual factors is essential in order to minimize tissue and function loss, as well as to reduce discomfort and need of further medical/surgical intervention [24]. Also, it is known that local and systemic measures to control the colonization of the surgical wound at the early healing phase, associated with reduction of the infection's spreading, may increase the predictability of the results [25]. Tetracyclines have been proved to be effective in several bone- and joint-related infections [26–28].

Regarding bone regeneration strategies, the association of a biomaterial and a therapeutic agent that might induce bone formation while preventing bacterial infection could, undoubtedly, contribute to a more predictable clinical outcome. In this way, the objective of this research was to evaluate the proliferation and functional activity of osteoblast-induced human bone marrow cells, cultured on the surface of HA and Bonelike®, in the presence of therapeutic concentrations of doxycycline and minocycline.

2. Materials and methods

2.1. Preparation of samples

Bonelike® was prepared with the chemical composition of $65P_2O_5–15CaO–10CaF_2–10Na_2O$ in mol.% from reagent grade chemicals using conventional glass-making techniques. The composite was obtained by adding the milled glass to HA powder to 2.5 wt.%, using isopropanol as a solvent. The powders were then dried and sieved to less then 75 μm under a nitrogen atmosphere. Disc samples were therefore prepared for in vitro testing by uniaxial pressing at 200 MPa using steel dies to obtain 12 mm diameter discs. The discs were then sintered at 1300 °C (using a ramp rate of 4 °C min^{-1}), with the temperature maintained for 1 h, followed by natural cooling inside the furnace. Phase identification and quantification was assessed by X-ray diffraction and Rietveld analysis. X-ray diffraction (XRD) was performed on Bonelike® samples using a Siemens D5000 diffractometer with Cu Kα radiation ($\lambda = 1.5418$ Å). The scans were made in the range of 25–35° (2), with a step size of 0.02° and a count time of 2 s $step^{-1}$.

Detailed description of Bonelike® preparation has been previously reported [20].

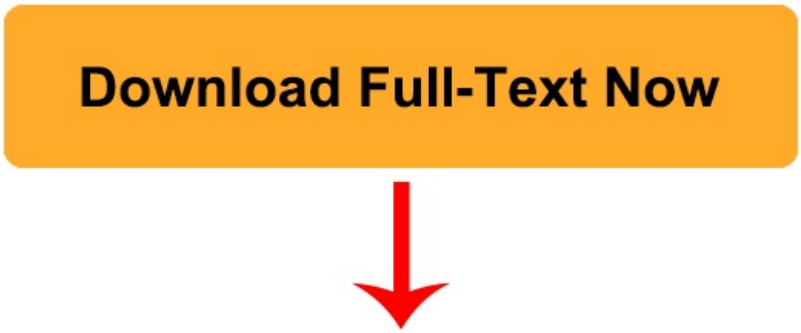
For in vitro testing, discs were mechanically polished to the same final topology of 1 μm using silicon carbide paper, ultrasonically degreased, cleaned with ethanol followed by deionised water and finally sterilized, prior to cell culture.

HA samples were also prepared as 12 mm diameter discs in order to compare their biological behavior with that of Bonelike®.

2.2. Cell culture

Human bone marrow was obtained from orthopedic surgical procedures conducted in adult patients (aged between 25 and 45 years). Informed consent was obtained for the use of this biological material, which would otherwise have been discarded. Bone marrow was cultured in α-MEM culture medium containing 10% fetal bovine serum, 50 μg ml^{-1} gentamicin, 2.5 μg ml^{-1} fungizone and 50 μg ml^{-1} ascorbic acid. Primary cultures were maintained in a humidified atmosphere (5% CO_2 in air at 37 °C) for 10–15 days until sub-confluence was reached. At this stage, cells were released enzymatically (0.05% trypsin and 0.025% collagenase) and the resultant suspension was cultured at a density of 10^4 cell cm^{-2}, in the culture medium described previously, further supplemented with 10 mM β-glycerophosphate and 10 nM dexamethasone. Cell cultures were established for 35 days and maintained on the surface of the culture plate (control cultures), HA or Bonelike® in the absence or presence of doxycycline or minocycline (1 μg ml^{-1}). Tetracyclines were both renewed at every medium change, which occurred twice a week.

ID	Title	Pages
2321	Cell-induced response by tetracyclines on human bone marrow colonized hydroxyapatite and Bonelike®	8



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