DEVELOPMENT AND TRIBOLOGICAL CHARACTERISATION OF MATERIALS FOR THE REPAIR AND REGENERATION OF ARTICULAR CARTILAGE

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Cartilage, hydrogels, bacterial cellulose, tribology

INTRODUCTION

The self regeneration of articular cartilage defects is quite seldom a spontaneous process, given its avascular nature and the reduced mitotic activity of cartilaginous cells (chondrocytes)[1]. In healthy individuals, articular joints may maintain their functionality for 60 years or more, yet about 90% of the world population with 70 years of age is affected by arthritis, with or without pain[2]. Surgical techniques[3] have aimed at alleviating pain, restoring function or delaying the progression of the affection. In the affected cartilage, the natural surface smoothness grows scarce[4-5] and friction coefficient values, normally as low as 0.003[6] may be higher. General surface morphology changes[7] leads to further degradation, wear and eventually to joint immobilization, thus being of the utmost importance the early detection of such lesions. Cartilage problems may remain unnoticed for years and solutions might require the use of total or partial prosthetic replacements. These solutions do fulfil their purpose but are limited, particularly in the case of young and active patients. A large number of materials has been proposed to repair cartilage defects, such as carbon fibre[8], calcium phosphates[9], periosteum[10] or polymeric hydrogels[11-13]. More recently, although very few studies have focused on the tribological behaviour under experimental conditions[13,14,15], tissue engineering has pushed forward the advancements in a wide range of materials to be used as three-dimensional scaffolds, focusing on the understanding and improvement of the chondrocytes' affinity for these 3D matrices[16-24].

MAIN GOAL

As aforementioned, the research to find compatible materials to repair degraded cartilage has lead to a myriad of materials to be pointed out as possible substitutes, among them, the hydrogels. This work has a two-fold objective – one the one hand, a substitute material for damaged articular cartilage is proposed: a synthetic polymer hydrogel that could present an immediate solution other than the current auto- or allografting; on the other hand, **a** tissue engineering approach is followed, using a natural polymeric hydrogel as scaffold where chondrocytes are expected to generate extracellular matrix (i.e.,cartilage) *in vitro*. In both cases, the tribological behavior is assessed in reciprocating sliding tests against bovine cartilage by characterisation of the wear mechanisms observed on the mating surfaces, as well as by the friction values obtained.

EXPERIMENTAL

Synthetic hydrogels

Blends of Poly(2-hydroxyethyl methacrylate) (PHEMA) and poly(methyl methacrylate) (PMMA) hydrogels (HG) where synthesised in a Teflon mould into pins. To the monomers in solution 2,2-azobisisobutyronitrile was added as the initiator. Polymerisation was carried out at 83 ºC, and several PHEMA to PMMA ratios were utilised.

Natural hydrogels

Bacterial cellulose (BC) pellicles secreted by *Gluconacetobacter xylinum* bacteria cultured in 24-well plates; BC disks were afterwards washed, autoclaved and kept in sterile conditions for cell-seeding. To promote cell adhesion, BC pellicles were functionalised by means of protein adsorption.

Tribological tests

The synthetic HG pins were tested using a pin on flat configuration, in reciprocating sliding against bovine articular cartilage (BAC). For each HG formulation, friction coefficient and wear rate were averaged. The latter was calculated based on mass loss determination – a scale was used for the synthetic HGs, and for the BC the phenol-sulphuric acid total glucose determination was used; the morphology of the mating surfaces was analysed by SEM. All tests had fixed parameters (stroke length, frequency and test duration, of 8mm, 1 Hz and 2 hours, respectively), whereas the normal load varied from 5 to 50 N, under lubricating conditions with phosphate buffered saline (PBS, Sigma-Aldrich) at room temperature and at 37 ºC. Sheets of BC samples were also tested varying the normal applied load (5 - 30 N) and test duration (0.5 – 6 hrs), at a temperature of 37 ºC. For each set of experimental parameters, a sliding test of self-mated BAC was performed, to serve as control.

Cell culture

A commercial adult human mesenchymal stem cell (hMSC) line was expanded *in vitro* in Dulbecco's Modified Eagle's Medium (DMEM) and incubated in a 5% CO_2 atmosphere, at 37 ºC. Functionalised BC pellicles were inoculated with hMSC and cell adhesion and viability were monitored at different time points by spectrophotometric measurement of metabolic activity by fluorescence of a staining solution.of DMEM and 10% resazurin (7-Hydroxy-3H-phenoxazin-3-one 10-oxide).

RESULTS AND DISCUSSION

Tribological tests pointed out that synthetic HGs with high content of PMMA (50% or higher), cause severe destruction of the cartilage surface. The higher the content in PMMA, the less water is absorbed and the hydrogel is quite rigid and brittle. On the other hand, the higher the starting HEMA content, the better the water absorption, hence a rubbery, compliant however mechanically resistant hydrogel has produced interesting results in terms of friction and wear. Friction values for dissimilar tribocouples averaged between 0.02 and 0.05, which compared to the self-mating cartilage surfaces results (0.01 – 0.02) demonstrates the potential use of such materials for the envisaged application. The wear rate of HGs with higher content in HEMA (90%), sliding against cartilage averaged $1.33x10^{-5}$ gm^{-1},whereas for HGs with lower content in HEMA (50%), the wear rate reached $3.54x10^{-4}$ gm^{-1}.
Analysing the pair BC/BAC surface morphology, BC showed an evident plastic deformation of the nanofibers, yet the entanglements apparently prevent the release of material into solution. That fact explains the difficulty in quantifying the wear rate by the phenol-sulphuric acid method. The BAC surface remained in most of the cases, smooth, but some deformation tracks are ascertainably observed.

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