Bone and gut microbiota crosstalk: A novel 3D *in vitro* approach

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Abstract

The present research aimed at shedding light on the interplay between the composition of the human gut microbiota and bone cells.

Introduction

The gut microbiota produces metabolites that maintain the homeostasis of the gut and contribute to the fitness of the host.[1] Recent reports showed a complex connection between the gut and bone health: during intrauterine and early postnatal life, the exposure or restriction of the environmental factors controls the growth, bone mineralization, and gut microbial composition.[2] The intimate association between the gut microbiota and skeletal metabolic processes suggests that the study of gut microbiota could have features for great clinical potential, encouraging the development of innovative diagnostic and therapeutic modalities.

In vitro models represent exploited valid alternatives to clinical or animal model studies for monitoring microbial composition.[3,4] Even if cell cultures are nowadays used to model complex interactions in vitro, gut microbiota culture remain a great challenge due to the great variety in the microbial composition that resides in the gut.

Here we developed a 3D *in vitro* model of the human gut microbiota, culturing the microorganisms on an electrospun gelatine structure. Then, in order to assess the gut-microbiota interplay, the products of intestinal cells obtained from the microbiota supernatants transiting were tested on osteoblasts cells.

Materials and Methods

The electrospun structures were fabricated starting from a gelatin solution crosslinked with (3-Glycidoxypropyl)-trimethoxysilane (GPTMS). Faecal samples were collected following the European guidelines for faecal microbiota transplantation. Scaffold mechanical and physical properties were assessed. The microbial behaviour within the Electrospun Gelatine (EG) structures was quantitatively and qualitatively analysed by Scanning Electron Microscopy (SEM), real-time qPCR, and Next Generation Sequencing (NGS) techniques. Human intestinal epithelial cell line Caco-2 and human osteoblast-like cells Saos-2 were used for biological experimentation. Viability and morphofunctional analyses were performed on both cell populations.

Results

Gelatin structures were suitable for supporting the adhesion and growth of the faecal microbiota. Scanning electron microscopy showed that the faecal microbiota adhered to the gelatin structures forming a multi-layered stable biofilm that persisted for at least 7 days (Figure 1).

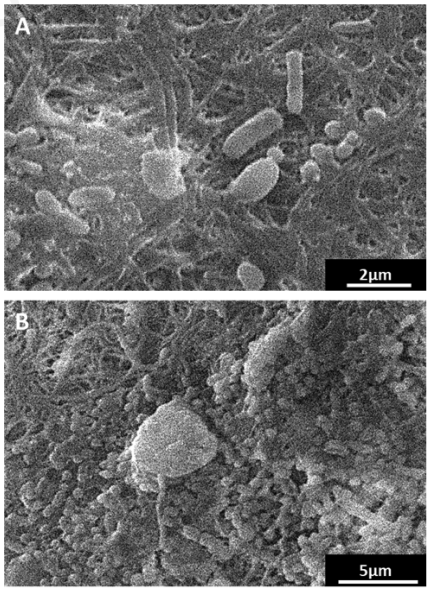

Figure 1. SEM micrograph of electrospun scaffolds with the faecal microbiota at 24 h (A), and 7 days (B) post-inoculation.

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Caco-2 cell viability was assessed with different dilutions of the 3D gut microbiota supernatant for up to 48 hours (Figure 2). The 1:2 and 1:5 dilutions were also used to evaluate changes in the cytoskeletal organization of epithelial cells. The media obtained from Caco-2 was then used to estimate viability and morphofunctional changes in Saos2.

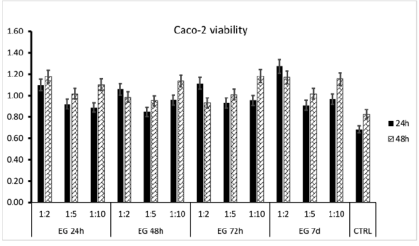

Figure 2. Caco-2 viability after incubation for 24h and 48h with diluted supernatants isolated from faecal microbiome grown on electrospun gelatin scaffold (EG) and collected at 24h, 48h, 72h and 7d from inoculum.

Discussion and Conclusions

Overall, our data indicate that the three-dimensionality of the structure-adhered microbial consortia maintain the bacterial biodiversity of the original sample, especially in the early stages of *in vitro* culture. These results demonstrate the validity of our system for *in vitro* culturing the human gut microbiota. Microbial assessment, as well as preliminary morphofunctional analyses on epithelial and osteoblastic, are encouraging for the development of a bioengineered platform to evaluate the microbiota crosstalk with bone tissue. The further step of our research will be the development of a human bone tissue model fluidically connected with the 3D gut microbiota through the interposition of an intestinal epithelial models.

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