Numerous efforts are going to develop functional scaffold to regeneration and bone repair application. Scaffolds that combine the bioactivity of the hydroxyapatite (HA) and the adjustable degradability of the polyurethane (PU) matrix obtained from the Poly(vinyl alcohol) (PVA) were developed in this research and submitted to morphological and biological characterizations. This new kind of scaffold is non toxic, has interconnected pores and microporous at the wall, great mechanical resistance and great cellular growing activation. These properties are essential to the clinical use requirements. In vitro and in vivo assays were performed to determined biocompatibility of PVAI-PU/HA scaffolds and analyze by scanning electron microscopy (SEM). Firstly, for in vitro assay, NIH3T3 cells were cultured for 7 days under scaffolds. A dense and continuous layer of fibroblasts under and inside of macroporous and microporous of scaffold were observed (Figure 1B and 1C – arrows). Cells covered by spreading themselves most of the outer surfaces of scaffold, due to the presence of lamellipodia and filopodia (Figure 1D - arrowheads). The PVAI-PU/HA promoted a great template for cell migration and spreading in vitro. For in vivo assays, PVAI-PU/HA discs were implanted in the subcutaneous tissue of Wistar rats for 24 hours, 7 and 14 days. After 24 hours of insertion (Figure 2), the biomaterial PVAI-PU/HA presented adherent cells, probably fibroblast, spreading forming compact and homogeneous cellular layer. Groups with PVAI-PU/HA inserted for 7 days, evidenced that cell adhesion colonization and infiltration were strongly affected by macro-architecture of the scaffold pore structure (Figure 3B-C). It was also observed an intense interlaced fibrous network formation inside of macroporous (Figure 3D). Finally, the implants maintained for 14 days presented fibrous capsule surround and inside of scaffold (Figure 4A and 4C). The scaffolds interact directly with tissue compounds growing through the scaffold's interconnected pores, without causing any inflammatory or rejection process (Figure 4B and 4D). Therefore, the PVAI-PU/HA scaffolds improving cell adhesion, showing greater biocompatibility and indicating promising expectations to osseous implants.

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Fig. 1: SEM of NIH3T3 cells cultured for 7 days in PVAI-PU/HA scaffold. A. Scaffold general view. B. Scaffold general view with NIH3T3 cells. C. Macroporous details showing tightly attachment of NIH3T3 cells with dense and continuous layer formation (asterisk). D. Details of cells spreading themselves outer surfaces of scaffold.

Fig. 2: SEM of the PVAI-PU/HA scaffolds into subcutaneous space for 24 hours. A. Scaffold general view. B. Details from inset in A. Observe cell migration and spreading (thin arrows) and early layers formation (arrows) in the scaffold. C. Cells suggestive of fibroblasts recovery all the scaffold surface.

Fig. 3: SEM of the PVAI-PU/HA scaffolds into subcutaneous space for 7 days. A. Scaffold general view. B. Details from 1A. Cells suggestive of fibroblast with extensive spreading ability. C. Details from 2A. Cells with high spreading ability (arrow). D. Details from 3A. Structures like collagen fibers. IN: internal area; * external area; C: fibrous material

Fig. 4: SEM of the PVAI-PU/HA scaffolds into subcutaneous space for 14 days. A. Scaffold general view. B. Details from A. Prolonged structures like collagen fibers. C. Scaffold general view. Innermost connection with tissue. D. Details from C. Invasion process of fibrous inner into scaffold. C: fibers; BM: biomaterial; CL: fibrous layer; CC: corneous layer.