High resolution silica tissue replica as cell culture scaffold for the differentiation of human mescenchymal stem cells

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Statement of Purpose: The complex 3D tissue microenvironment plays important roles in regulating cell functions and differentiation. In particular, topographical information harboured within the native tissue niches is known to instructive cues that can guide the differentiation of mesenchymal stem cells (MSC) (Tse et al., 2011, Park et al., 2011, Tang et al., 2014). We have recently shown that such topographical information can be replicated on biomaterials by a process known as bioimprinting (Tong et al, 2010). However, this method can only replicate the topography of tissue niches on the surface of a 2D cell culture substrate, which is devoid of the 3D complexity of a microenvironment. Here, we report the replication of 3D extracellular environment by utilizing teramethyl orthosilicate hydrolysis. This process has been used as a sample preparation technique in scanning electron microscopy (SEM) to preserve the nanoscale structure of cells (Kaehr et al., 2012) and tissues (Townson et al., 2014). Here, we explored the use of this technique to generate silica replicas of native tissues that can be used as cell culture scaffold. We propose this method as a fast and convenient way of producing in vitro niches for MSC differentiation.

Methods: Native tissues sections (bovine: achilles tendon and spinal cord; Sus: cartilage and muscle) were fixed with paraformaldehyde and incubated with teramethyl orthosilicate in acidic condition, followed by dehydration and calcination to remove the majority of organic matters. The resulting silica tissue replicas were examined by bright field microscopy and SEM. To examine the potential differentiation guidance capability of the replicas, human bone marrow derived MSC were cultured (Minimum Essential Medium Alpha Medium supplemented with 15% heat inactivated fetal calf serum and 1% antibiotic-Antimycotic) on to a silica replica of bovine spinal cord for 21 days. The expression of neurogenic markers SOX1, ENO2 and beta-tubulin III were examined by real time-PCR.

Results: SEM images (Figure 1) show that the silica replicas are almost identical in ultrastructure to native tissues. Our real time-PCR data (Figure 2) shows the expression of neurogenesis markers, SOX1 and ENO2, was higher in MSC cultured for 21 days on the silica replica of bovine spinal cord than in those cultured for the same duration on a flat glass surface (SOX1: 1.98 fold; ENO2: 2.83 fold).

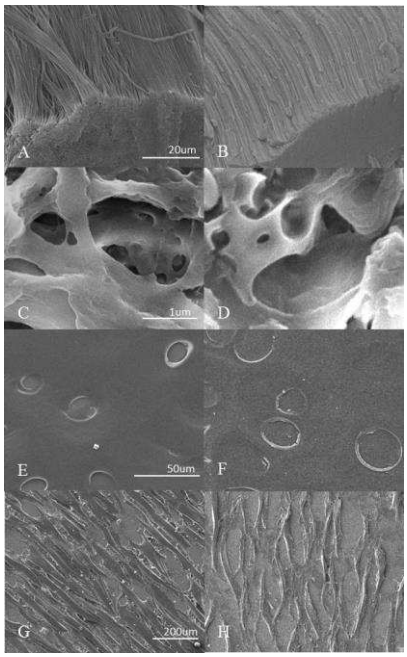

Figure 1: SEM images (A) Native tendon section (B) Silica replica of tendon (C) Native spinal cord (D) Silica replica of spinal cord (E) Native cartilage (F) Silica replica of cartilage (G) Native muscle (H) Silica replica of muscle

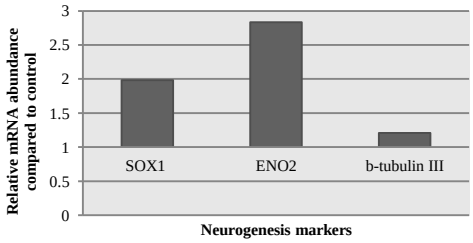

Figure 2: Relative mRNA abundance of neurogenesis markers *SOX1, ENO2 and β*-tubulin III compared to control (i.e. glass slide) after 21 days incubation on silica replica of spinal cord

Conclusions: Silicification of native tissue is a fast and simple method to generate high resolution tissue replicas. Silica spinal cord replica retains sufficient topography information that was able to guide mescenchymal stem cells differentiating towards the neuronal lineage without any differentiation factors in the medium. The future study will be focus on testing the differentiation guidance using different tissue replicas.

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