Abstract Details

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Abstract title:

Improving three-dimensional in vitro culture methods of human endometrial stem cells: bioengineering tissue-specific hydrogels

Biography

Universitary Degree in Biotechnology. University of Pablo Olavide, Seville, Spain. (2012-2016)
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Study question:

Can tissue-specific extracellular matrix (ECM) hydrogels derived from decellularized (DC) porcine endometrium improve three-dimensional (3D) culture of human endometrial stem cells (ICE6-7, Cervelló et al.,2011)?

Summary answer:
ECM hydrogels from DC porcine endometrium are biocompatible and improve ICE6-7 proliferation compared to standard 3D culture, showing much potential for future regenerative medicine purposes.

What is known already:
The use of hydrogels made of tissue-specific ECM from DC organs represents a revolution in regenerative medicine due to its therapeutic potential. This is attributable to the presence of bioactive components that mimic the natural tissue-specific environment, together with low immunogenicity. Recently, our group has established a method to decellularize whole pig uteri (Campo et al.,2017). Here we furthered this line of investigation using the DC organ to create endometrial ECM hydrogels. These were used in novel *in vitro* approaches and will be tested in the near future *in vivo* as a novel solution for untreatable endometrial pathologies.

Study design, size, duration:
Pig uteri (n=4) were collected for whole organ decellularization, endometrium isolation and hydrogel creation. Physicochemical features were determined and compared with hydrogels from a non-DC endometrium and DC myometrium. Biocompatibility of DC endometrial hydrogels (at 3, 6 and 8 mg ECM/ml) were studied using stem cell lines from epithelial (ICE6) and stromal (ICE7) origins (n=3) during 24 and 72hrs in different conditions. Standard matrix for 3D culture, collagen and Matrigel, were used as controls.

Participants/materials, setting, methods:
To create hydrogels, uteri were decellularized by perfusion and endometrium was isolated by microdissection. Subsequently, DC endometrial tissue was milled, lyophilized and enzymatically

digested. The characterization was carried out by turbidimetry, scanning electron microscopy (SEM), quantification of ECM proteins and proteomic analysis. ICE6 and ICE7 cell lines were cultured on top of (2.5D) or embedded in (3D) hydrogels and cell proliferation was measured by MTS Assay. Two-way Anova was used for statistical analysis.

Main results and the role of chance:
While decellularization removes much of the protein fraction (81%), a significant amount of elastin (18%) and glycosaminoglycans (18%) was retained. Moreover, a significant enrichment of collagen (50%) was observed. SEM showed that endometrial ECM hydrogels are porous scaffolds with a homogenous, randomly interlocking fibrillar structure. No significant differences in fiber thickness (0.10 µm) and ultrastructure were found between non-DC endometrial, DC myometrial hydrogels and different concentrations. Turbidimetry showed that stable endometrial ECM hydrogels quickly formed after incubation at standard culture conditions (12.97±1.46 min at 37ºC), independently of the concentration. After 72hrs, cell proliferation of both cell lines was improved in 3D culture system with endometrial ECM hydrogel (all concentrations) compared to collagen and Matrigel controls. A fold change of up to 2.13 and 2.11 (6 mg/ml $p<0.0001$) compared to collagen, for ICE7 and ICE6 respectively was measured. In 2.5D culture, collagen and Matrigel poorly retained the cells compared to the endometrial hydrogels. Here, a fold change of up to 1.84 and 1.83 (8mg/ml, $p<0.0001$) for ICE7 and ICE6 respectively was observed. These data showed that porcine endometrial ECM hydrogels are biocompatible and improve *in vitro* proliferation of human endometrial stem cells.

Limitations, reasons for caution:
This study was only performed with endometrial stem cell lines, *In vivo* studies will be required to confirm the regenerative potential of endometrial ECM hydrogels under pathological conditions.

Wider implications of the findings:
These findings support the hypothesis that porcine endometrial ECM hydrogels are biomimetic to the native endometrium and stem cell niche. This study represents a first step to the use of this biomaterial to improve 3D *in vitro* culture, opening a window towards *in vivo* studies and treatment of endometrial pathologies.

Trial registration number:
not applicable

Keywords:
bioengineering
stem cells
3D culture
endometrium
extracellular matrix hydrogels