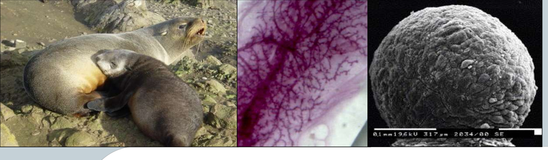

Matrigel impedes normal development of Cape fur seal primary mammary cells, possibly through the activation of the TGFß pathway.

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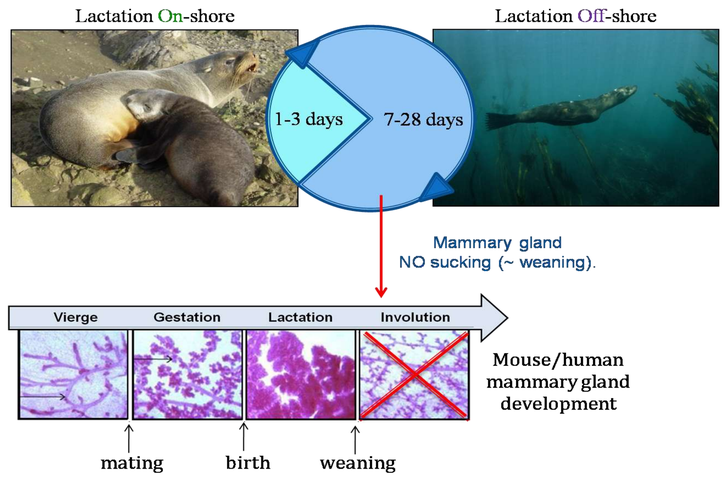
✓ Introduction

Cape fur seals belong to the Pinnipedia family and, as all sea mammals, give birth and nurse their pup on-shore but rapidly need to go off-shore, where the food is. These animals have evolved and adopted different lactation strategies, to manage this temporal and spatial problem. While some such as the true seals (Phocid sub-family), have adopted a fasting strategy, consisting of feeding continuously their pup on-shore, others (otariid sub-family) including the Cape fur seal, have adopted a foraging strategy with interrupted lactation as their body is not big enough to store bubbler layer to remain on-shore for a long period of time without eating. Thus, the Cape fur seal feeds its pup only for few days periods before foraging alone at sea for up to 4 weeks. Once the bubbler replenishes, the female returns on-shore to produce milk again. This lactation cycle is carried out for 10 months[1]. Interestingly, during the foraging time, the mammary gland does not enter into the involution process despite the absence of sucking, which normally induces the signalling of programmed death of the epithelial cells in all the other mammalian species, bringing a return of the mammary gland to a virgin-like stage. This ability to switch milk production "on/off" makes this animal a unique model for the study of mammary gland biology, especially for the transition of lactation to involution[2]. Here, we present the development of an *in vitro* seal mammary gland cell culture model.



✓ Results

1. Cape fur seal primary mammary cells form mammospheres on plastic but develop stellate-like structures on Matrigel.

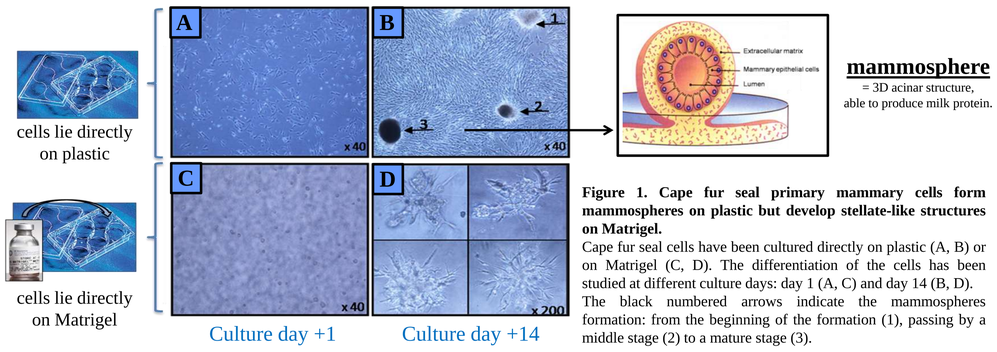


Figure 1. Cape fur seal primary mammary cells form mammospheres on plastic but develop stellate-like structures on Matrigel.
Cape fur seal cells have been cultured directly on plastic (A, B) or on Matrigel (C, D). The differentiation of the cells has been studied at different culture days: day 1 (A, C) and day 14 (B, D). The black numbered arrows indicate the mammospheres formation: from the beginning of the formation (1), passing by a middle stage (2) to a mature stage (3).

Matrigel™, a gelatinous protein mixture secreted by mouse sarcoma, is a commonly used biomatrix for the growth and differentiation of bovine and mouse primary mammary cells into mammospheres; a 3-dimensional acinar structure resembling the secretory alveolus in the mammary gland. In contrast, these cells only grow as a monolayer and do not differentiate when cultured on plastic[3,4]. Surprisingly, our previous results showed that seal primary mammary cells behave in a totally opposite way from mouse or bovine cells in culture. When cultured on plastic the seal cells form mammospheres and develop a stellate-like phenotype on Matrigel.

2. Matrigel overrides the signals of seal cells differentiation

To check if the stellate-like structures observed on Matrigel are due to the incapacity of individual cells to recognize various signals of differentiation, differentiated seal mammospheres (grown initially on plastic) were transferred onto Matrigel. Interestingly, this promotes a switch to stellate-growth after few days in culture, suggesting that Matrigel can override the differentiated mammosphere organisation.

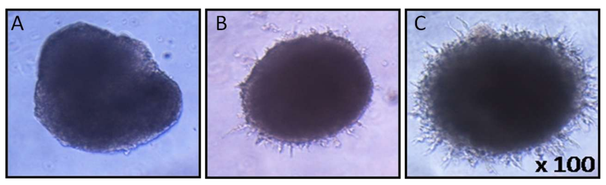


Figure 3. Matrigel overlaps the cell-cell interactions of seal mammosphere grown on plastic, turning it to a stellate-like structure.
Evolution on Matrigel of seal mammospheres at different culture days: day 1 (A), day 5 (B) and day 11 (C).

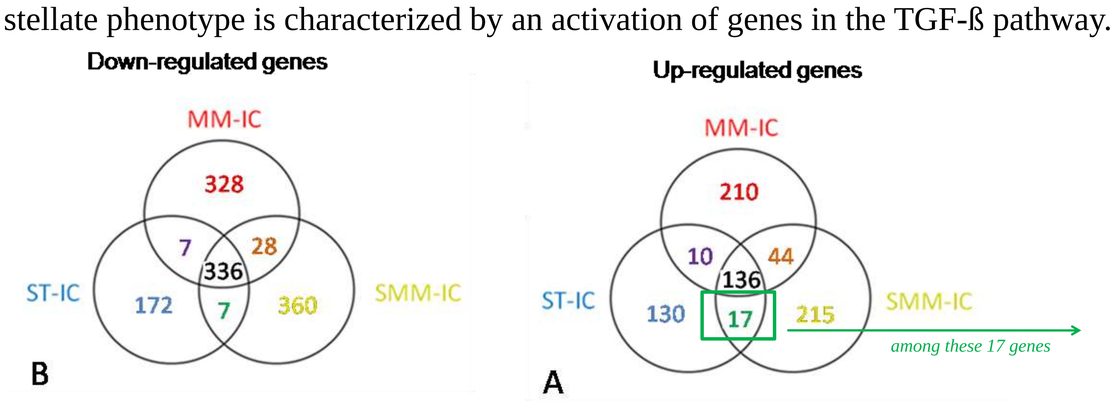
3. Seal primary mammary cells do form mammospheres on collagen I, supposing a unique effect of Matrigel

Cape fur seal do form mammospheres on collagen I from rat tail.
Mammosphere formation from seal primary mammary cells when cultured on collagen after 4 days in culture.

To see if seal cells do develop a stellate-phenotype on any type of exogenous matrix, we cultured them on collagen I from rat tail, which is a commonly used matrix for mouse or bovine primary mammary cells in cell culture. The results show that the seal cells on collagen do form mammospheres after few days in culture, suggesting that the stellate-like phenotype is specific to Matrigel.

4. Microarray analysis on non-differentiated seal cells (individual seal cells on plastic), differentiated cells (mammospheres) and stellate structures.

To understand the developmental differences observed when seal cells are grown on plastic compared to Matrigel, Affymetrix arrays were performed on individual seal cells (used as t=0) (IC), stellate cells (ST), mammospheres (MM) and stellate-like mammospheres (SMM). A seal array not being available yet and the dog being the evolutionary closest animal to the seal, a canine Affymetrix array was used. Through this analysis, many genes have been observed up-regulated in both ST and SMM, so specifically in the stellate phenotype. Interestingly, we found that among these genes LTBP3 and TGF-β1, all members of the TGF-ß pathway. The results were validated by RT-QPCR. The data shows that the stellate phenotype is characterized by an activation of genes in the TGF-ß pathway.



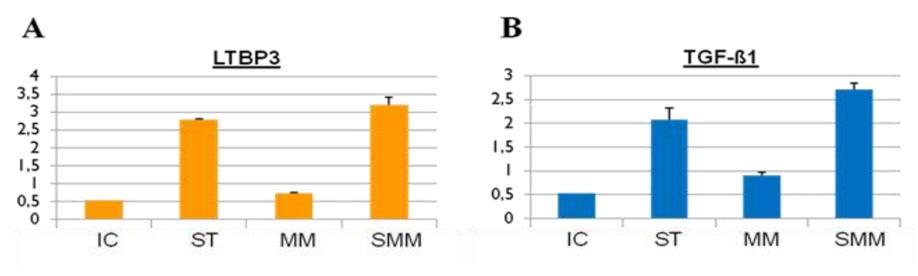


Figure 5 - Venn diagram showing the distribution of the genes up-regulated (A) or down-regulated (B) in mammospheres (MM), stellates (ST) and Stellate-like mammospheres (SMM) related to the genes expressed in individual cells (IC), considered as the reference t=0. (41393 EST are present in this canine microarray).

RT-PCR quantitative analysis on LTBP3 (A, C), TGF-β1 (B, D) on individuals cells (IC), stellates (ST), mammospheres (MM) and stellate-like mammospheres (SMM) from female 1 (A, B) and female 2 (C, D). The expression of these genes has been normalized by GAPDH expression.

✓ Conclusion and perspectives

The aim of our study was to examine the characteristics of seal primary mammary cells cultured on plastic (their own matrix) compared to Matrigel in order to better understand the extracellular matrix mediated signaling that leads to correct cellular polarization and mammosphere formation. To do so, we conducted microarray analyzes on non-differentiated seal cells (individual seal cells on plastic), differentiated cells (mammospheres) and stellate structures. The results show that the stellate phenotype is characterized by an activation of genes from the TGF-ß pathway, well known for its role in cell development, migration and matrix formation[4]. Surprisingly, these results are in contrast with data from the mouse model in which the TGF-ß pathway was up-regulated in mammary cells on plastic, presumably to stimulate the cells to produce a matrix required for mammosphere formation[4]. The results suggest that Matrigel impedes normal development of seal mammary cells resulting in a loss of polarization which is probably due to an epithelial-mesenchymal transition known to be stimulated by TGF-ß pathway. Thus, cape fur seal represents a new model to study the specificity of the matrix in normal cellular development and better understand cell-matrix interactions and signaling, mediating cell polarization and differentiation.

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