

Effects of Tamoxifen and oestrogen on histology and radiographic density in high and low mammographic density human breast tissues maintained in murine tissue engineering chambers

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Abstract

Purpose

Mammographic density (MD) is a strong risk factor for breast cancer. It is altered by exogenous endocrine treatments, including hormone replacement therapy (HRT) and Tamoxifen. Such agents also modify breast cancer (BC) risk. However, the biomolecular basis of how systemic endocrine therapy modifies MD and MD-associated BC risk is poorly understood. This study aims to determine whether our xenograft biochamber model can be used to study the effectiveness of therapies aimed at modulating MD, by examine the effects of Tamoxifen and oestrogen on histologic and radiographic changes in high and low MD tissues maintained within the biochamber model.

Methods

High and low MD human tissues were precisely sampled under radiographic guidance from prophylactic mastectomy fresh specimens of high-risk women, then inserted into separate vascularized murine biochambers. The murine hosts were concurrently implanted with Tamoxifen, oestrogen or placebo pellets, and the high and low MD biochamber tissues maintained in the murine host environment for three months, before the high and low MD biochamber tissues were harvested for histologic and radiographic analyses.

Results

The radiographic density of high MD tissue maintained in murine biochambers was decreased in Tamoxifen-treated compared to oestrogen-treated mice (p=0.02). Tamoxifen treatment of high MD tissue in SCID mice led to a decrease in stromal (p=0.0088), and an increase in adipose (p=0.023)

percent areas, compared to placebo-treated mice. No histologic or radiographic differences were observed in low MD biochamber tissue with any treatment.

Conclusion

High MD biochamber tissues maintained in mice implanted with Tamoxifen, oestrogen or placebo pellets had dynamic and measurable histologic compositional and radiographic changes. This further validates the dynamic nature of the MD xenograft model, and suggests the biochamber model may be useful for assessing the underlying molecular pathways of Tamoxifen-reduced MD, and in testing of other pharmacologic interventions in a preclinical model of high MD.

Keywords

Mammographic density, Tamoxifen, oestrogen, stroma, xenograft model, bioengineering chambers

Introduction

Mammographic density (MD) is the area of radiologically white or bright tissue seen on a mammogram [1,2]. High MD adjusted for age and body mass index (BMI) is one of the strongest risk factors for breast cancer (BC), with a relative risk of four to six times comparing the highest to the lowest MD quartile [1]. Prospective studies of Tamoxifen have demonstrated a reduction in the risk of a new primary breast cancer in patients receiving adjuvant endocrine therapy, and also among women at high risk for breast cancer due to a strong family history [3,4]. More recently, studies have shown that women receiving adjuvant Tamoxifen after BC surgery who had a reduction in MD derived the greatest risk reduction in breast cancer prevention [5,6]. Meanwhile supplemental oestrogen in the form of HRT increases MD of post-menopausal women [7,8], which is reduced on withdrawal of the HRT preparations [8].

To better understand the effects of systemic endocrine changes on alterations in MD and its associated malignancy risk, a preclinical model to define how Tamoxifen and oestrogen can modulate MD and BC risk will be beneficial. Such a preclinical model would also be useful for testing other treatment options that arise for reducing MD. We have previously validated a xenograft model of human MD where high and low MD human breast tissues were propagated within paired biochambers based on the epigastric pedicle of SCID mice [9,10]. Immunohistochemical staining confirmed the persistence of human tissues in these biochambers, and the histologic and radiographic MD phenotypes of the biochamber tissues were preserved relative to the original human breast tissues [9]. The high and low MD biochamber tissues also responded in a dynamic manner to alterations in the murine systemic hormonal milieu of pregnancy, lactation and involution [10].

To determine if this model can be used to effectively investigate how Tamoxifen and oestrogen modulate MD and breast cancer risk, we have assessed the effects of exogenous endocrine therapies on histologic and radiographic parameters in our biochamber model. High and low MD tissues from human breast samples were maintained in the biochamber model for three months, with the murine hosts treated with Tamoxifen, oestrogen or placebo. The histologic and radiographic changes observed in the harvested biochamber MD tissues may assist in clarifying the cellular changes due to endocrine interventions and how this mediates BC risk.

Method

Accrual of high and low MD regions from within the same breast

Approval from the Peter MacCallum Human Research Ethics Committee (#08/21) and St. Vincent's Hospital Animal Ethics Committee (049/09) was obtained for MD tissue samples from ten high-risk women undergoing prophylactic mastectomy. The mammograms of all participants were examined by the study radiologist and the BIRADS category noted. Radiographic-guided sampling of high and low

MD regions from within the same fresh mastectomy specimen was performed by the slice biopsy method as previously described [11,9].

The high and low MD tissues were kept viable and sterile on ice, then separately partitioned for murine biochamber propagation, histologic (H+E or IHC staining of formalin-fixed, paraffin-embedded tissues or tissues mounted in OCT) and radiographic analyses, as well as future analysis (snap frozen in liquid nitrogen).

Maintenance in murine biochambers together with exogenous Tamoxifen, Oestrogen or Placebo implants

High and low MD samples were minced, mixed 2:1 with Matrigel (BC Biosciences, Bedford, MA) supplemented with FGF-2 (1 μ g/ml), Sigma-Aldrich, Sydney, Australia), and then separately transferred into silicone biochambers implanted in the groins of SCID mice, based on the right and left epigastric pedicles respectively [9,10].

At the time of biochamber implantation, the SCID mice also were implanted with either Tamoxifen (free base) (25mg/pellet, 60 day release, Innovative Research of America, Sarasota Florida), oestrogen (17 β -estradiol, 1.7mg/pellet, 90 day release, Cat NE-121, Innovative Research of America) or placebo pellets (Placebo for Tamoxifen (free base), 25mg/pellet, 60 day release, Innovative Research of America) in a subcutaneous pocket made in the dorsal cervical region [12]. The mice bearing MD tissues from the same woman were randomly assigned to different treatment groups (per Supplementary Fig. 1).

Material from the biochambers was harvested at three months post-insertion for histologic and radiographic examination. In event of murine urosepsis as a complication of high-dose oestrogen, the affected mice were treated with antibiotics in their drinking water, and biochamber material harvested early if the host mouse was sacrificed early due to sepsis. Urosepsis is a common side effect observed in oestrogen-treated SCID mice [13], thus is an exclusion criteria for quantitative analysis of high and low MD biochamber tissues if the tissues examined on microscopy were affected by severe inflammatory infiltrate and fat necrosis.

Histologic analysis of biochamber tissues

Paraffin-embedded tissue sections (4 μ m) of high and low MD biochamber tissues were stained with H+E and then examined histologically with digital microscopy (AxioVision/Zeiss photomicroscope), to determine the percent areas of stromal, glandular and adipose tissues.

IHC staining for oestrogen receptor (ER) (Ventana, Clone SP1, Cat. #790-2223) was performed to examine if there was a difference in hormone receptor staining between high and low MD tissues

maintained in murine hosts implanted with Tamoxifen, oestrogen or placebo pellets. ER nuclear staining was assessed by separately point-counting the nuclear staining of epithelial and stromal cells, expressed as a percentage of total ER positive cells, in high and low MD tissues of the three treatment groups. IHC staining for Vimentin (V9) (DakoCytomation, Clone V9, Code M0725) was performed to examine the distribution of stromal and adipose cells in high and low MD biochamber tissues in the three treatment groups [14]. Pairs of high and low MD biochamber tissues were stained with Masson's Trichrome Blue (MTB) for assessment of collagen in the three treatment groups, then quantitative assessment performed, including blinded assessor scoring of the intensity of blue collagen staining in high and low MD biochamber tissues of the three treatment groups, on a scale of 1 to 6 (with 1- 2 for weak staining, 3 – 4 moderate staining, and 5 - 6 strong staining).

Images of the stained tissue sections were imported into image analysis software, JMicroVision v1.2.7 (Geneva, Switzerland), for threshold-masking to quantify absolute and percent areas of the histologic components from the total area, and ER point counting, as previously described [9,10].

Radiographic analysis of biochamber tissues

The high and low MD biochamber tissues harvested from murine hosts implanted with Tamoxifen, oestrogen or placebo pellets were examined with specimen x-ray imaging at St Vincent's BreastScreen (0.0017dGy, 23 keV, 40mAs)[11,9]. A calibration strip comprising exponentially stepped transparency sheets was used with each film [9]. Matched pairs of high and low MD biochamber tissues were cut to the same thickness (2 mm) before radiographic imaging was performed.

Image analysis software, J MicroVision v1.2.7, was used to determine the mode of signal intensity for each biochamber core compared to the signal intensity of the calibration strip [9].

Statistical analysis

Normality was assessed with D'Agostino and Pearson Omnibus normality test prior to determining statistical significance using GraphPad Prism 5.0 (CA, USA). A one-way ANOVA test was used to compare across more than two groups of data, with post-hoc comparison performed to identify which two comparisons resulted in the difference across the three groups. To determine if a difference was observed between high compared to low MD biochamber tissues in each treatment group, the ANOVA test with post-hoc comparison, and a paired t-test were performed separately. To compare the radiographic densitometry between two groups of MD biochamber tissues, the t test was used for parametric data, and Mann-Whitney test for non-parametric data. The p-value of significance for rejecting a null hypothesis was 0.05.

Results

Study sample

The study sample consisted of high and low MD biopsies from within-individual breasts of ten high-risk women undergoing prophylactic mastectomy at St Vincent's Hospital Melbourne. The women were at increased risk of malignancy due to a strong family history of BC or having a BRCA1/2 gene mutation, or a past history of BC. The demographic characteristics of the study women are listed in Tables 1 and 2.

Murine hosts in study sample

Six of 28 mice in the oestrogen group and seven of 31 mice in the tamoxifen group were sacrificed between 2 to 3 months due to urosepsis, and these high and low MD biochamber tissues were excluded from quantitative histologic or radiographic assessment after microscopic examination for presence of significant chronic inflammatory infiltrate or fat necrosis (15% of mice). One mouse in the oestrogen group and two mice in the tamoxifen group had mild urinary tract infections, and the biochamber tissues did not demonstrate fat necrosis or inflammatory changes, thus were included for analysis (3% of mice). The 27 mice in the placebo group were healthy throughout the study duration. Thus high and low MD biochamber tissues from 24 tamoxifen-treated mice, 22 oestrogen-treated mice and 27 placebo-treated mice were included for statistical analysis.

Descriptive histologic changes of biochamber tissues with Tamoxifen or oestrogen supplementation

H+E sections of high MD biochamber tissues harvested from mice implanted with placebo pellets demonstrated abundant stromal tissue, with small amounts of glandular and adipose tissue (Fig 1a-c), while low MD biochamber tissues from mice implanted with placebo pellets demonstrated abundant adipose tissue, moderate stromal and small amounts of glandular tissue, in keeping with previous findings (Fig 1d-f) [9,10].

In mice implanted with Tamoxifen pellets, H+E sections of high MD biochamber tissues demonstrated moderate amounts of stromal and adipose tissue, as well as medium-sized ducts and small lobules (Fig 1a), while H+E sections of low MD biochamber tissues demonstrated abundant adipose tissue with few small glands and stroma (Fig 1d).

In mice implanted with oestrogen pellets, H+E sections of high MD biochamber tissues demonstrated abundant stromal tissue, with small amounts of adipose and small glands (Fig 1b). H+E sections of low MD biochamber tissues harvested from mice implanted with oestrogen demonstrated moderate amounts of adipose and stromal tissues, with small amounts of glandular tissue (Fig 1e).

Quantitative analysis of biochamber tissues with Tamoxifen, oestrogen or placebo supplementation

Quantitative analysis of the percentage area of stromal tissue in high MD samples showed that Tamoxifen-supplemented tissues were significantly decreased compared to placebo-supplemented mice (ANOVA, post hoc test p-value=0.0088, Fig 2a). Similarly, there was an increase in the percentage area of adipose tissue in high MD biochamber tissues treated with Tamoxifen, compared to placebo (ANOVA, post hoc test p-value=0.023, Fig 2b). No difference was observed in the glandular percentage area of high MD biochamber tissues maintained in mice implanted with Tamoxifen, compared to placebo (ANOVA, p=0.3, Fig 2c). There was no significant effect of oestrogen treatment compared to placebo on histologic composition within high MD tissue (Figs 2a-c).

There was no difference observed in the stromal (ANOVA, p=0.78), adipose (ANOVA, p=0.68) or glandular tissue percentage areas (ANOVA, p=0.78) of low MD biochamber tissues maintained in murine hosts implanted with Tamoxifen, oestrogen or placebo pellets (Fig 3a-c).

Radiographic changes of biochamber tissues with Tamoxifen, oestrogen or placebo supplementation

In keeping with our previous work, there was increased radiographic density of high compared to low MD biochamber tissues in the placebo group (Mann-Whitney test, p=0.031, Fig 4a). We also observed an increased radiographic density comparing high to low MD biochamber tissues harvested from mice implanted with oestrogen (t test, p=0.0015, Fig 4b). No difference was observed in high compared to low MD biochamber tissues in Tamoxifen-treated mice (Mann-Whitney test, p=0.18, Fig 4c). The radiographic density in high versus low MD biochamber tissues was different across the three treatment groups (two-way ANOVA, post hoc test p-value<0.0001), with the difference observed between high and low MD biochamber tissues in oestrogen-supplemented mice (Fig 5).

A difference in radiographic density was observed between the high MD human breast tissues treated with Tamoxifen- compared to oestrogen-supplemented mice, with decreased radiographic density (ANOVA, post hoc test p-value=0.02, Fig 6a). However no difference was observed in low MD biochamber tissues maintained in Tamoxifen-, oestrogen- or placebo-treated mice (ANOVA, p=0.37, Fig 6b).

ER staining in glandular tissues

ER nuclear staining occurred predominantly in glandular epithelium, and to a lesser extent in the stromal cells (Fig 6). There was no difference in quantitative point counting of total ER nuclear staining between high (ANOVA, p=0.24) or low MD biochamber tissues (ANOVA, p=0.67) harvested from mice implanted with Tamoxifen, oestrogen or placebo pellets (Fig 7). However, a difference was observed in the percentage ER staining of epithelial cells in the high MD biochamber tissues of the three treatment groups, due to decreased percentage ER nuclear staining in epithelial cells of oestrogen- compared to tamoxifen-treated mice (ANOVA, post hoc test p-value=0.036). There was no difference in percentage ER staining of epithelial cells in low MD biochamber tissues (ANOVA,

p=0.53), or stromal cells in high (ANOVA, p=0.94) or low MD biochamber tissues (ANOVA, p=0.37, (Fig 8a-d)).

Collagen staining in high and low MD biochamber tissues

MTB staining of high and low MD biochamber tissues harvested from mice treated with Tamoxifen, oestrogen and placebo demonstrated a circumferential distribution of collagen fibres around glandular structures (Fig 9). MTB staining in high and low MD biochamber tissues of Tamoxifen-treated mice was suggestive of decreased intensity of collagen staining, compared to oestrogen- and placebo-treated mice (Fig 9). However no difference was seen in the quantitative MTB staining intensity (intensity scores) of high (ANOVA, p=0.8368) and low MD biochamber tissues (ANOVA, p=0.3136) between the three treatment groups (Fig 10).

Vimentin staining in stromal and adipose MD biochamber tissues

V9 staining of high and low MD biochamber tissues harvested from mice treated with Tamoxifen, oestrogen and placebo pellets demonstrated brown staining of stromal and adipose cells of human origin (Fig 11), in keeping with our previous findings [9]. The stromal tissues were distributed circumferentially around glandular tissues in all three treatment groups (Fig 11).

Discussion

MD, BC risk and Tamoxifen-induced changes

High MD is one of the strongest risk factors for BC, independent of other known risk factors for the disease [1,2]. How MD is modified by endogenous and exogenous hormonal alterations in a woman at a cellular level is not fully understood. Meanwhile the biocellular changes responsible for the risk reduction associated with MD modification in Tamoxifen-treated women are still unknown and require further study at a preclinical level. Using our xenograft model of human MD, we show that high MD human biochamber tissue is altered by Tamoxifen treatment; including a reduction in stromal and an increase in adipose percent areas (Figs 1-2). Similarly we demonstrated that the radiographic density of high MD biochamber tissues was decreased in Tamoxifen-treated mice (Fig 6a).

In contrast, there was no difference in the histologic composition or radiographic density of low MD human breast tissues maintained in the biochambers of mice supplemented with Tamoxifen, oestrogen or placebo (Figs 3a-c, 6b), demonstrating that the effects observed in high MD tissues was not seen in low MD tissues. The observation that high compared to low MD biochamber tissues harvested from mice implanted with placebo pellets had increased radiographic density is in keeping with our previous studies that examined high and low MD biochamber tissues in this xenograft model of human MD, further validating the robustness of this model [9,10].

Our results suggest that the decrease in radiographic density observed in high MD human breast tissues implanted in biochambers of mice treated with Tamoxifen is associated with a decrease in stromal and glandular areas histologically. Whether the breast cancer risk reduction may be due to a reduction in volume of glandular and stromal tissues at risk, or caused by a functional alteration in the stromal and epithelial cells themselves, or changes in the interaction between the stromal and epithelial compartments, warrants careful examination in further studies.

Tamoxifen supplementation has been shown to bind ER, thus inhibiting the effects of oestrogen in breast tissues, and oestrogen supplementation down regulates ER, relative to placebo [15]. In keeping with this, we observed a difference in percent ER nuclear staining in epithelial cells of high MD biochamber tissues harvested from Tamoxifen-, oestrogen- or placebo-treated mice in this study, with a reduction in percent ER staining of oestrogen- compared to Tamoxifen-treated epithelial tissues (Fig. 8a).

High MD does not only reflect the amount of fibroglandular and adipose tissue, but is also affected by other cellular fractions and connective tissues in the breast, which in turn may alter BC risk. Changes in stromal collagen composition and structure have previously been found to increase MD and BC risk [16,17]. Other studies suggested that high extracellular matrix (ECM) content, a perpendicular alignment and cross-linking of collagen fibres, may increase MD and breast cancer risk [16,18,19]. Such ECM and collagen factors can also be investigated in this MD xenograft model. MTB staining of high and low MD biochamber tissues in mice treated with Tamoxifen, oestrogen and placebo demonstrated circumferential distribution of collagen fibres around glandular structures, in keeping with previous findings [9]. V9 staining of high and low MD biochamber tissues in the three treatment groups also demonstrated a circumferential distribution of stromal cells of human origin around glandular structures (Fig 11). To answer the question regarding the underlying mechanism of decreased radiographic density in the Tamoxifen group fully, other studies of cell-type specific RNA analysis for ECM factors and ECM degradation enzymes will be useful and could be performed in the future.

Several studies demonstrate that Tamoxifen or oestrogen supplementation in tumour growth studies can affect tumour growth in MFP [20-23]. Other studies have shown that Tamoxifen administered by subcutaneous injection or implanted in rats or mice can result in decreased estrogen receptor binding, with sufficient tissue perfusion to demonstrate increased tissue ER activation, and changes in end organs [24,25]. Furthermore, our previous work on the effects on murine peripartum lactation, involution and pregnancy upon high and low MD human mammary biochamber tissues have demonstrated that the biochamber tissue changes with alterations in endogenous hormone levels, suggesting that the mammary tissues in the biochambers are responsive and affected by systemic hormonal changes [10].

Meanwhile an increased percent area of adipose tissue was observed in the high MD biochamber tissues of Tamoxifen-treated mice. If the increase in the percentage area or volume of adipose tissue in the breast caused by Tamoxifen is mainly due to increased maturation of stromal cells into adipocytes, with concurrent decreased radiographic density, this may be another potential avenue for reduction in MD-associated risk that warrants future investigation.

The low MD biochamber tissues did not alter in histologic composition or radiographic density during maintenance in Tamoxifen, oestrogen or placebo-supplemented murine hosts. This may be because the effects of the endocrine agents are more manifest in high compared to low MD breast tissues. Other possible explanations are that there was an insufficient duration of Tamoxifen or oestrogen supplementation to result in MD modification, or the sample size was insufficient to observe histologic or radiographic differences in low MD biochamber tissues.

MD, BC risk, ER and Oestrogen-induced changes

The radiographic density of high MD biochamber tissues maintained in mice implanted with oestrogen was increased over the three month duration, when compared to that of high MD biochamber tissues in mice supplemented with Tamoxifen (Fig 6a), but not compared to placebo-treated high MD biochamber tissues. This is in keeping with published literature regarding MD and exogenous hormonal therapies showing that MD is increased in women taking HRT, and may be reduced in women who are taking Tamoxifen. [26-31]

In our study, low MD biochamber tissues were not affected by oestrogen supplementation, where both the radiographic density and histologic percent areas of stroma, glandular and adipose tissues were unchanged. No difference was observed in the histologic composition in high MD biochamber tissues of mice supplemented with oestrogen, when compared across the three hormonal groups. This may be due to a smaller effect size of oestrogen on MD characteristics in breast tissues, compared to combined oestrogen and progesterone hormonal supplementation, as reflected by large scale cohort studies on long-term HRT [26-28].

Limitations of the study

A limitation of the study was that a proportion of mice developed sepsis from urinary tract infections due to prolonged high-dose oestrogen and Tamoxifen implants. Antibiotic water is often used in estrogen supplementation studies in immunocompromised mice to avoid issues associated with high estrogen dosing such as urosepsis. A 1.7mg/90day release pellet was used in this study, which gave a significant estrogen supplementation, but did lead to some mice developing urinary tract infections, treated with antibiotics. Those mice with urosepsis resulting in severe fat necrosis and chronic inflammation within the biochamber tissue samples (15%) were excluded from analysis in the study. Nevertheless the results from this study were able to demonstrate significant differences in histologic

composition and radiographic density in high MD biochamber tissues affected by the difference in systemic endocrine environment due to tamoxifen, oestrogen or placebo implants.

Conclusion

High MD biochamber tissues maintained in mice implanted with Tamoxifen, oestrogen or placebo pellets had measurable radiographic and histologic changes in the percentage stromal and adipose areas, further validating the dynamic nature of the MD xenograft model. This suggests that the biochamber model may be useful for testing of other pharmacologic interventions or novel biomolecular agents in a preclinical model of MD.

Competing interests

The authors declare that they have no competing interests.

Acknowledgments

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Figure legends

Fig. 1 The tissue sections of high and low MD biochamber tissues harvested from SCID mice implanted with Tamoxifen, oestrogen or placebo pellets stained with H+E. The tissue sections show the histologic qualitative appearance of high MD biochamber tissues of (a) Tamoxifen-treated, (b) oestrogen-treated, (c) placebo-treated mice, and low MD biochamber tissues of (d) Tamoxifen-treated, (e) oestrogen-treated, (f) placebo-treated mice. HD – high MD, LD – low MD

Fig. 2 The histologic composition of high MD biochamber tissues harvested from mice implanted with Tamoxifen, oestrogen or placebo pellets. Data shows percent areas of (a) stromal (b) adipose and (c) glandular tissues. Data is represented as scatter plot column graphs with significance levels denoted by * $p < 0.05$, ** $p < 0.01$. Tam – tamoxifen, E2 – estrogen, Plac – placebo, Str – stroma, Gln – gland, HD – high MD.

Fig. 3 The histologic composition of low MD biochamber tissues harvested from mice implanted with Tamoxifen, oestrogen or placebo pellets. Data shows percent areas of (a) stromal (b) adipose and (c) glandular tissues. Data is represented as scatter plot column graphs. Tam – Tamoxifen, E2 – estrogen, Plac – placebo, Str – stroma, Gln – gland, LD – low MD.

Fig. 4 The radiographic density of high compared to low MD biochamber tissues harvested from mice implanted with Tamoxifen, oestrogen or placebo pellets. Data shows the radiographic density of high versus low MD biochamber tissues harvested from (a) Placebo-treated, (b) oestrogen-treated and (c) Tamoxifen-treated SCID mice, in relative densitometry units. Data is represented as scatter plot column graphs with significance levels denoted by * $p < 0.05$, ** $p < 0.01$. Tam – Tamoxifen, E2 – estrogen, Plac – placebo, HD – high MD, LD – low MD.

Fig. 5 The radiographic density of high and low MD biochamber tissues harvested from mice implanted with Tamoxifen, oestrogen or placebo pellets. Data shows the radiographic density of (a) high MD and (b) low MD biochamber tissues in relative densitometry units. Data is represented as

scatter plot column graphs with significance levels denoted by * $p < 0.05$. Tam – Tamoxifen, E2 – estrogen, Plac – placebo, HD – high MD, LD – low MD.

Fig. 6 The tissue sections of high and low MD biochamber tissues harvested from mice implanted with Tamoxifen, oestrogen or placebo, stained for oestrogen receptor. The tissue sections show the histologic qualitative appearance of high MD biochamber tissues of (a) Tamoxifen-treated, (b) oestrogen-treated, (c) placebo-treated mice, and low MD biochamber tissues of (d) Tamoxifen-treated, (e) oestrogen-treated, (f) placebo-treated mice. HD – high MD, LD – low MD

Fig. 7 The total ER staining of high and low MD biochamber tissues harvested from mice implanted with Tamoxifen, oestrogen or placebo pellets. Data shows quantitative point counting of total ER nuclear staining in (a) high MD, and (b) low MD biochamber tissues of Tamoxifen-, oestrogen- or placebo-treated SCID mice. Data is represented as scatter plot column graphs. Tam – Tamoxifen, E2 – estrogen, Plac – placebo, HD – high MD, LD – low MD.

Fig. 8 The percentage ER staining of epithelial and stromal tissues of high and low MD biochamber tissues harvested from mice implanted with Tamoxifen, oestrogen or placebo pellets. Data shows the percentage ER staining of ER+ epithelial cells (over total epithelial cells) in a) high MD biochamber tissues, and b) low MD biochamber tissues; and of ER+ stromal cells (over total stromal cells) in c) high MD biochamber tissues, and d) low MD biochamber tissues, of Tamoxifen-, oestrogen- and placebo-treated mice. Tam – Tamoxifen, E2 – estrogen, Plac – placebo, HD – high MD, LD – low MD.

Fig. 9 The tissue sections of high and low MD biochamber tissues harvested from mice implanted with Tamoxifen, oestrogen or placebo, stained with Masson's Trichrome Blue. The tissue sections show the histologic qualitative appearance of high MD biochamber tissues of (a) Tamoxifen-treated, (b) oestrogen-treated, (c) placebo-treated mice, and low MD biochamber tissues of (d) Tamoxifen-treated, (e) oestrogen-treated, (f) placebo-treated mice. HD – high MD, LD – low MD

Fig. 10 The Masson's Trichrome Blue staining intensity score of high and low MD biochamber tissues harvested from mice implanted with Tamoxifen, oestrogen or placebo pellets. Data shows the Masson's Trichrome Blue staining intensity score of a) high MD biochamber tissues, and b) low MD biochamber tissues, of Tamoxifen-, oestrogen- and placebo-treated mice. Tam – Tamoxifen, E2 – estrogen, Plac – placebo, HD – high MD, LD – low MD.

Fig. 11 The tissue sections of high and low MD biochamber tissues harvested from mice implanted with Tamoxifen, oestrogen or placebo, stained with Vimentin. The tissue sections show the histologic qualitative appearance of high MD biochamber tissues of (a) Tamoxifen-treated, (b) oestrogen-treated, (c) placebo-treated mice, and low MD biochamber tissues of (d) Tamoxifen-treated, (e) oestrogen-treated, (f) placebo-treated mice. HD – high MD, LD – low MD

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Tables

Table 1 Demographic characteristics of participants in the study, including their age, Birads category, family history, past history, menopausal status and parity

Demographic characteristics	Number, N, or Mean
Age	Mean 44.2 years (Range 34 - 55 years)
Birads category	
4	3
3	2
2	2
1	3
Family history	
Nil	3

BRCA-, strong family history	1
BRCA1	3
BRCA2	3
Menopausal status	
Pre	6
Post	4
Past history of breast disease	
Yes – BC or DCIS	6
No	4
Parity	
Parous	9
Nulliparous	1

BC – breast cancer, DCIS – ductal carcinoma in situ

Table 2 Results of operative specimens from study cohort, including operation performed and histology results

Participant	Operation performed	Histology result
1	Bilateral SSM – sample from right side	Right – MDE. Left – old scar, MDE. No malignancy
2	Bilateral SSM – sample from left side	Right – old scar. Left – FCC, MDE, ductal epithelial hyperplasia of usual type. No malignancy
3	Bilateral SSM – sample from left side	Right – MDE. Left – FCC. No malignancy
4	Right SSM – sample from right side	Right – focal adenosis and columnar cell change. No malignancy or DCIS
5	Bilateral SSM – sample from right side	Right – FCC, MDE. Left – FCC, MDE. No malignancy
6	Left SSM – sample from left side	Left – old scar, with small foci of intermediate grade cribriform DCIS in cavity of previous WLE scar, ER 85% PR 90%
7	Right SSM – sample from right side	Right – Normal breast tissue.
8	Bilateral SSM – sample from left side	Right – Normal breast tissue. Left – Normal breast tissue. No malignancy
9	Bilateral SSM – sample from right side	Right – MDE. Left – old scar, MDE. No malignancy
10	Bilateral SSM – sample from left side	Right – Normal breast tissue. Left – MDE. No malignancy

SSM – skin sparing mastectomy, MDE – mammary duct ectasia, FCC – fibrocystic change, DCIS – ductal carcinoma in situ, IDC – invasive ductal cancer

Figures

Fig 1

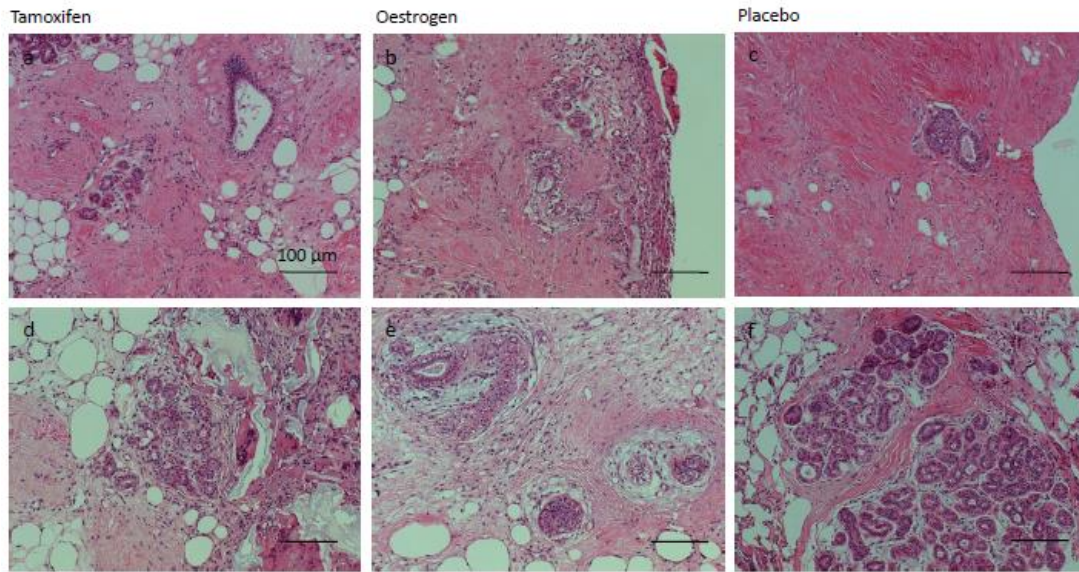


Fig 2

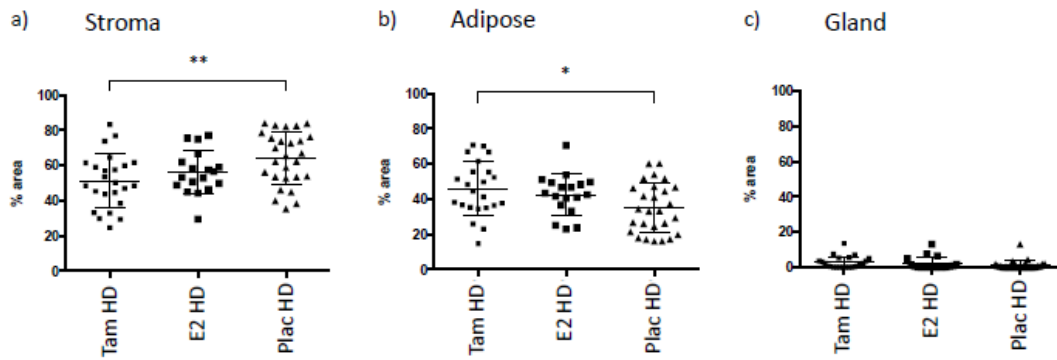


Fig 3

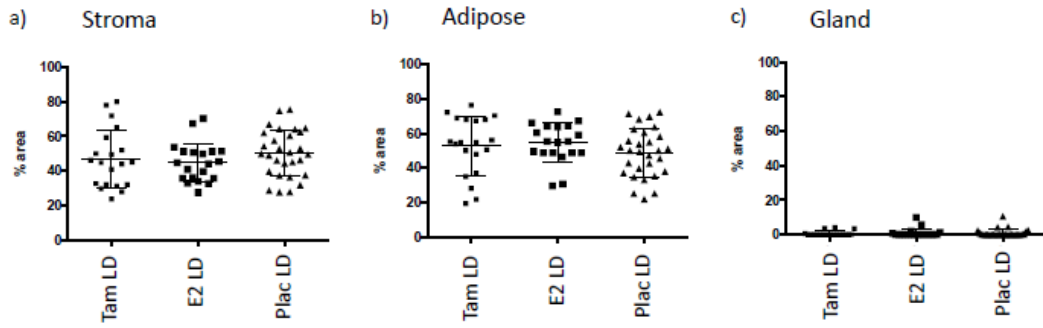


Fig 4

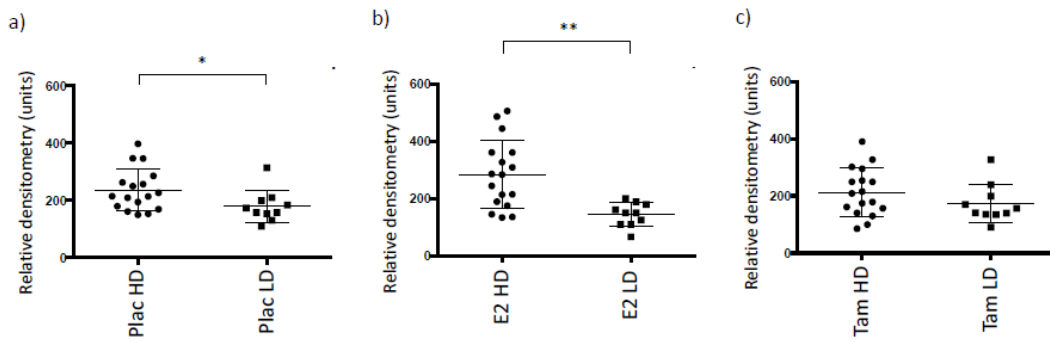


Fig 5

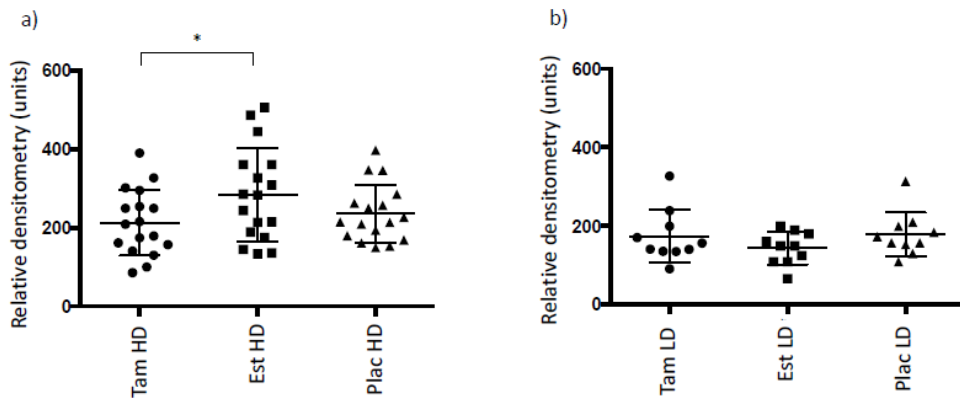


Fig 6

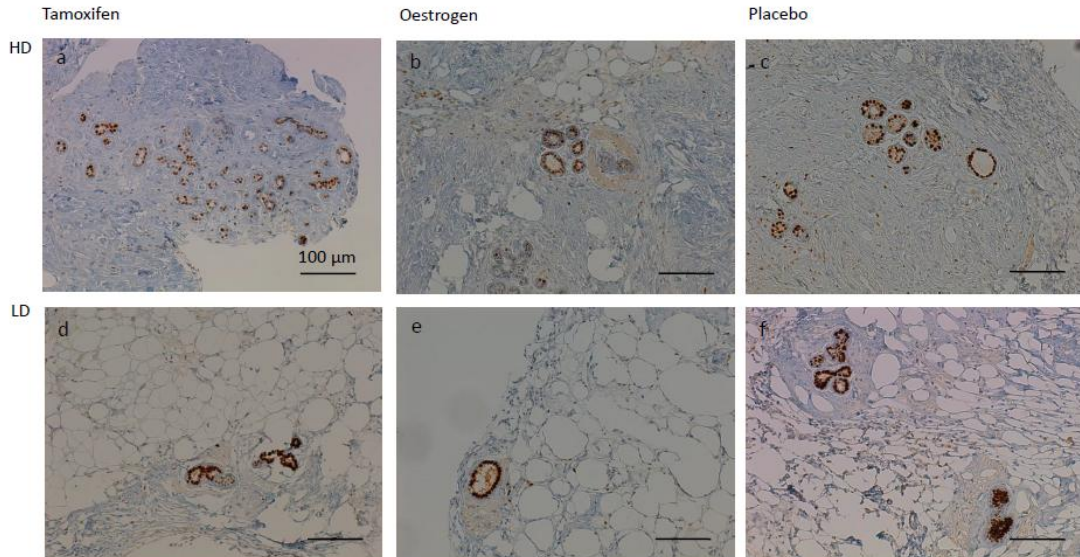


Fig 7

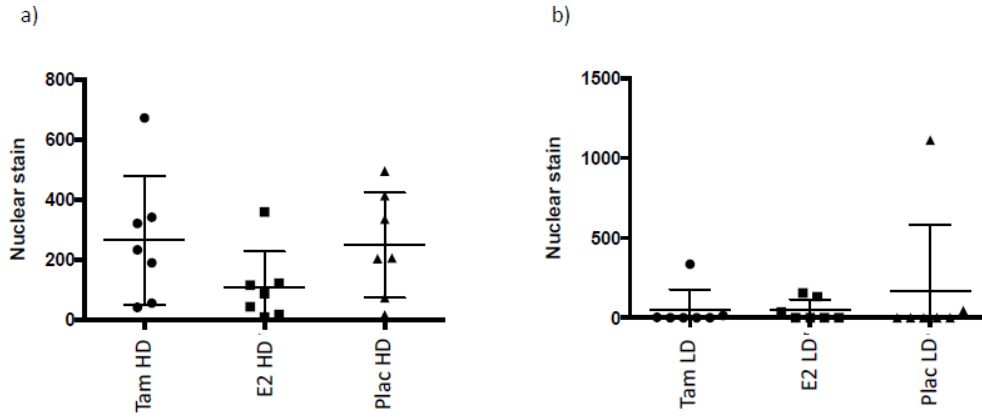


Fig 8

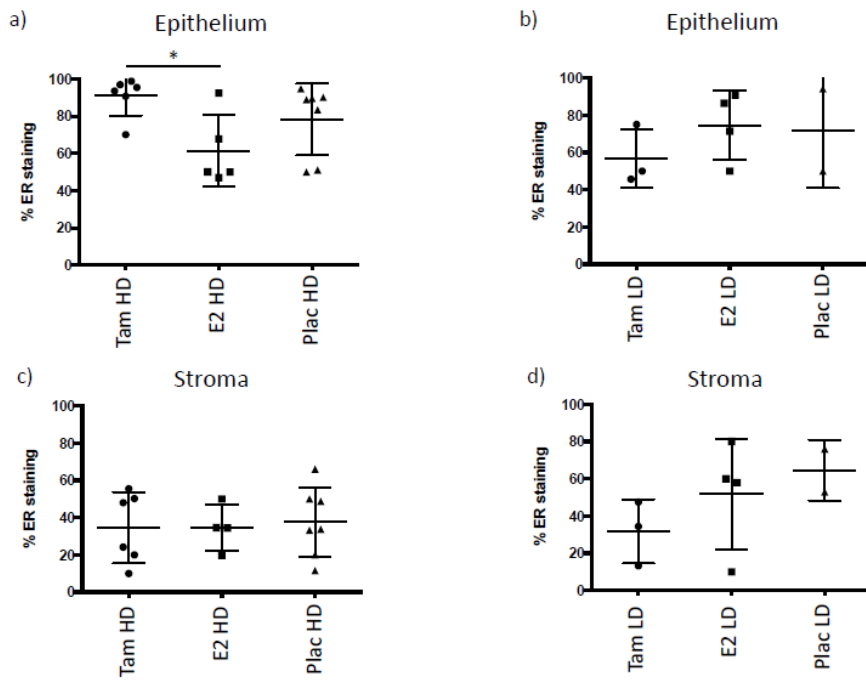


Fig 9

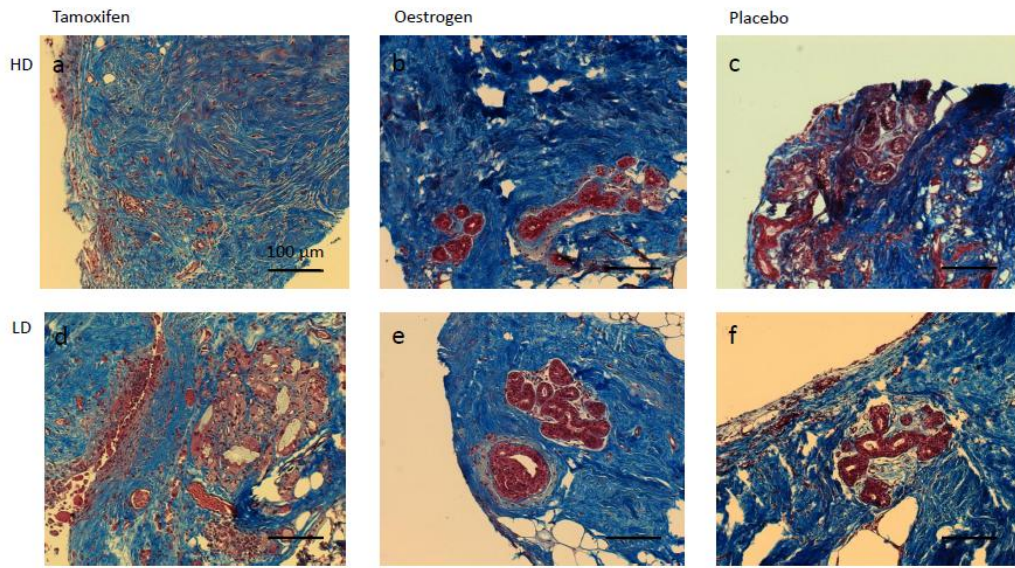


Fig 10

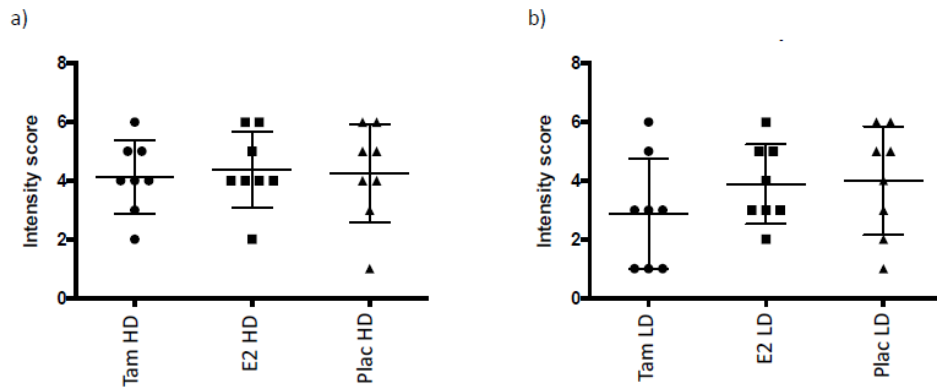
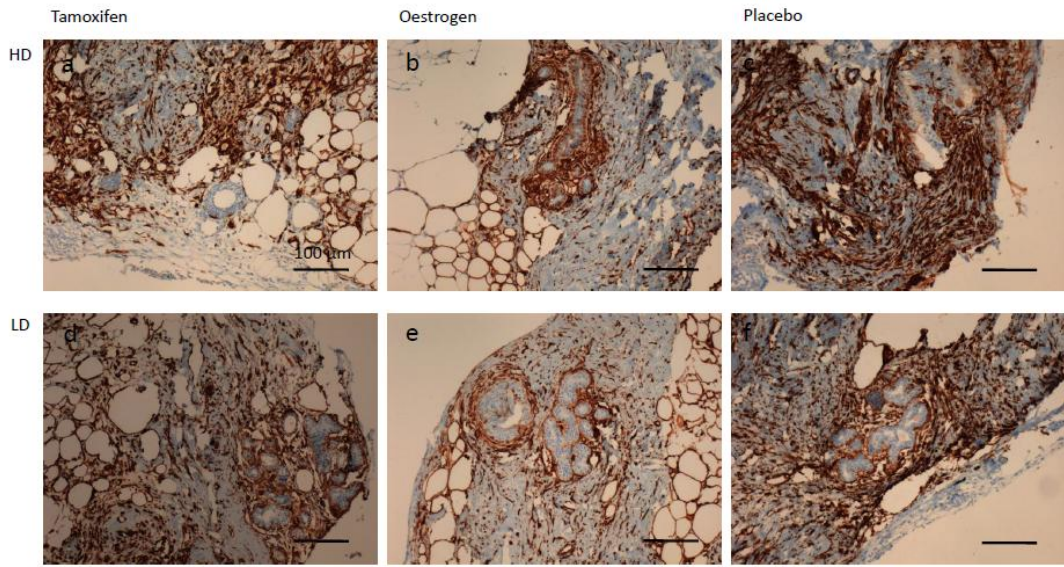


Fig 11





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