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Towards Effective Radiation-Induced Hypothyroidism Therapeutics: Identifying Potential Biomarkers for In Vitro Thyroid Scaffold Evaluation

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Abstract

Objective: Radiation-induced hypothyroidism (RIHT) affects up to 92% of head and neck cancer patients following irradiation, significantly impairing quality of life. Existing treatments show multiple limitations, but thyroid tissue engineering presents a promising alternative. Validated biomarkers for assessing thyroid scaffolds in vitro remain limited. This study identifies candidate protein markers in the Nthy-ori 3-1 cell line, informed by expression patterns in a murine RIHT model.

Methods: Immunofluorescent analysis of murine thyroids at days 3 and 28 post-irradiation identified preliminary protein candidates. An electrospun 10% (w/v) polycaprolactone scaffold was fabricated and characterized, then seeded with Nthy-ori 3-1 cells to monitor biomarker expression over 14 days.

Results: Imaging demonstrated reduced thyroxine in irradiated glands, confirming RIHT onset. Decreased E-Cadherin and increased α -smooth-muscle actin and fibronectin indicated fibrosis, while transient Collagen-1 reduction reflected extracellular matrix remodeling with reduced Zonula Occludens-1 (ZO-1) suggesting epithelial disruption. Within scaffolds, Nthy-ori 3-1 cells displayed a six-fold increase in DNA content, higher metabolic activity, and collagen-like fibril formation. Expression changes in α -smooth-muscle actin, fibronectin, ZO-1, and Collagen-1 mirrored irradiation-induced alterations but normalized by day 14, while increasing E-Cadherin and thyroid-specific proteins suggest emerging hormone-producing potential of the scaffold construct.

Conclusion: These findings identified key biomarkers for evaluating thyroid scaffold performance in vitro and support their relevance for RIHT tissue-repair strategies.

1 Introduction

Radiotherapy is a primary treatment modality for head, neck and breast cancer which together account for approximately 20% of malignant cancers worldwide [1, 2]. The thyroid gland, situated in the neck, is often inadvertently included in the radiation field, resulting in off-target injury of this endocrine organ and subsequent development of radiation-induced hypothyroidism (RIHT) which severely decreases patient quality of life. RIHT is characterised by a reduction of the thyroid hormones thyroxine (T4) and triiodothyronine (T3). Despite being estimated to affect up to 92% of patients who have undergone irradiation (IR) for head and neck or breast cancer, the pathophysiological mechanisms of this disease, particularly the point of disruption in the hormone synthesis pathway (**Figure1a**), remain poorly understood [3, 4, 5, 6]. Furthermore, the only conventionally available treatment, hormone replacement therapy, carries significant caveats with its use, including insufficient symptomatic relief and interaction with concomitant medication or dietary constituents [7].

Tissue engineered scaffolds have recently emerged as a prospective alternative therapeutic for RIHT [7, 8]. Among these, electrospun scaffolds provide great promise as compared to other fabrication techniques, electrospinning allows precise control over physical and mechanical scaffold properties [9, 10]. *In vitro* assessment of scaffold performance is crucial to advance the development of these therapies. This commonly includes investigation of functionally and structurally relevant proteins expressed by cells cultured on these scaffolds, such as hepatocyte albumin expression in the case of scaffolds for liver regeneration [11, 12]. However, partially due to under-characterization of protein expression changes during RIHT, as well as the lack of expression of functionally important proteins in commonly used thyroid cell lines, these biomarkers remain yet to be determined in the thyroid gland. This is further complicated by the recent discontinuation of a ‘gold-standard’ thyroid cell line FRTL-5, attributed to a divergence in karyotype [13].

Herein, an established murine model of head and neck radiation injury was utilized to explore the short- and long-term pathophysiology of RIHT *in vivo* and identify potential biomarkers for evaluation of therapeutics [14]. To assess *in vitro* efficacy of these biomarkers and further highlight the potential of tissue engineered therapeutics for RIHT, immortalized human thyroid cells were cultured on an electrospun polycaprolactone (PCL) scaffold and the expressional changes of potential biomarkers was investigated over multiple weeks.

2 Results

2.1 Thyroxine hormone levels significantly decreased following irradiation

We first aimed to characterise the localisation of thyroid hormone production in mouse thyroid gland. Using non-irradiated thyroid gland (D0) as an exemplar, it was evident that thyroxine (T4) localized primarily intracellularly within the thyrocyte membrane and, at high concentration, on the basement membrane of the follicular lumen (**Figure 1c**). Quantitative assessment of T4 expression demonstrated a significant decrease of the hormone in the thyroid gland both short- (3-days) and long-term (28-days) following IR, particularly in the follicular lumen (**Figure 1c & d**).

2.2 Irradiation disrupts thyroid follicle structures and extracellular matrix proteins

It is well established that irradiation injury causes disruption to epithelial architecture in other organs, such as the salivary glands and intestines [15, 16]. Histological imaging highlighted that there was also a significant disruption of thyroid structures post-IR. D0 tissue displayed closed follicles with the extracellular matrix (ECM) primarily located throughout the colloid (**Figure 2a**). In contrast, H&E images of day 3 post-IR revealed disruption of the follicle basement membrane and loss of cell polarity (green arrows), as well as loss of colloid ECM (blue arrows). This was substantiated by findings at day 28 post-IR where extensive breakdown of the follicle structures and a nearly complete lack of colloid structures was observed.

Additionally, IF analysis displayed an alteration in expression and localization of multiple extracellular matrix (ECM)-associated proteins. An interplay of expression of the cell-cell adhesion protein E-Cadherin (ECAD) and the contractile protein α -smooth-muscle actin (α SMA) was observed, with the former decreasing at day 3 and further at day 28 post-IR while α SMA showed the inverse pattern. α SMA, in particular, appeared to increase in areas which were abundant in ECAD in non-irradiated tissue, possibly indicating an inverse correlation (**Figure 2b & f**). Expression of collagen-1 (COL1) markedly reduced at day 3 and 28 post-IR, and localization changed from a widespread cover of follicle lining thyrocytes to a concentration around the outer thyrocytes (Figure 2C & 2F). The major ECM component fibronectin (FN) increased significantly short- and long-term post-IR, with no apparent change in localization (**Figure 2 d & f**). Localization of the tight junction protein zonula occludens-1 (ZO-1) remained consistent around the edges of the follicular lumen and extrafollicular junctions, regardless of IR. However, a significant decrease in ZO-1 levels was observed at day 28 post-IR, further confirming follicle membrane disruption (**Figure 2e & f**).

2.3 Response of thyroid-related functional proteins to irradiation varied

Interestingly, no significant effect was observed on thyroid-stimulating-hormone receptor (TSHR) and thyroid peroxidase (TPO) expression nor localization (**Figure 3a, c & d**). While the overall expression of the thyroid hormone synthesis substrate thyroglobulin (Tg) did not change, its localization was significantly altered. In non-IR tissue Tg localized within the follicular lumen but an evident change to intracellular localization within thyrocytes was noted at day 3 and day 28 post-IR (**Figure 3b & d**). Moreover, a continuous and significant increase of thyroid transcription factor 1 (also known as NK2 homebox 1, NKX2-1) expression was observed following IR.

2.4 Enhanced metabolic activity of Nthy-ori 3-1 cell cultured on electrospun scaffolds

In order to explore the response of thyroid cells in a 3D-bioengineered scaffold, Nthy-ori 3-1 cells were seeded on electrospun scaffolds and cultured for 14 days. During culture the cells displayed elevated metabolic activity with a significant increase evident over a 14-day period (**Figure 4b**). DNA content of the seeded scaffolds also increased over this period by an 8-fold factor during the first 7 days and a further, albeit non-significant, increase of 1.2-fold between 7 and 14 days of culture was noted (**Figure 4c**).

2.5 ECM protein expression of Nthy-ori 3-1 cells cultured on the scaffold was altered during study

SEM imaging of the cell-seeded scaffolds illustrated the ability of Nthy-ori 3-1 cells to attach to fibres and penetrate into pores of the scaffold, in addition to their ability deposit ECM (**Figure 5a**). No morphological changes indicative of cell death, such as smoothening or blebbing, [17] were observed. Relative expression of ECM proteins of Nthy-ori 3-1 cells cultured on the electrospun scaffold was assessed via IF analysis and normalized to the nuclei count. α SMA expression peaked at day 7 of study but declined at day 14, albeit remaining higher than at day 1 (**Figure 5b & f**). Furthermore, while it may appear that α SMA displayed co-localization with ECAD at day 1 and 7, this may be an artifact of the PCL scaffold diffracting the fluorescence. However, expression of ECAD significantly increased from day 1 to day 7 and persisted at this level to day 14 of culture (**Figure 5b & f**). Curiously, COL1 displayed the same trend in expression as α SMA (**Figure 5c & f**). FN expression remained unchanged throughout the study (**Figure 5d & f**). Expression of ZO-1 decreased at day 7 of study but returned to original levels by day 14 (**Figure 5e & f**).

2.6 Nthy-ori 3-1 cells culture on the scaffold displayed a steady increase in thyroid-specific protein expression

In line with analysis of ECM proteins, the changes in expression levels of thyroid-specific proteins of Nthy-ori 3-1 cell-seeded scaffolds was investigated through IF imaging over 14 days and normalized to the nuclei count. Expression of TPO, Tg, TSHR and NKX2-1 displayed a significant increase over the duration of study (**Figure 6**). Together, these data indicate that the scaffolds supported cell growth and functionality, *in vitro*.

3 Discussion

While the pathophysiology of RIHT remains to be elucidated, previous research has suggested several avenues, including induction of fibrotic processes, thyroiditis, apoptosis, atherosclerosis as well as the development of thyroglobulin autoantibodies [7, 18, 19, 20]. Through both immunofluorescent and H&E staining, significant disruption of the ECM was observed following thyroid IR, which is a known sign of and contributor to tissue fibrosis in other organs [21, 22, 23]. Specifically, a long-term increase in the ECM proteins α SMA, COL1, and FN was found in this study. IR has been shown to chronically activate fibroblasts, a cell type that functions to maintain normal ECM deposition, into myofibroblasts [24, 25]. This transition into myofibroblasts, which express high levels of α SMA, exhibit enhanced FN assembly and produce large quantities of COL1, is a primary mediator of tissue fibrosis [26, 27]. Additionally, a stark decrease in ECAD was observed following thyroid IR. Curiously, this is in direct contradiction to a previous study which indicated an increase in ECAD expression following IR of human squamous cell carcinoma of the thyroid gland, *in vitro* and *in vivo* [28]. However, it is important to note that the response of healthy and cancerous thyroid tissue to IR may differ. Moreover, multiple studies have suggested an involvement of epithelial-to-mesenchymal transition (EMT), associated with decreased ECAD expression, in fibrosis, albeit these studies are primarily limited to pulmonary fibrosis [29, 30]. Similarly to activation of fibroblasts, EMT is also known to occur as a result of IR [31, 32]. A key event of the EMT process is the loss of cell polarity and the reorganization of intercellular junctions, particularly tight-junctions [33, 34]. Both of these features were observed in this study in the thyroid gland following IR, with the loss of cell polarity evident in H&E images and the loss of tight junctions signified by the long-term decrease in ZO-1 expression. Taken together, this evidence suggests that IR causes fibrosis of the thyroid gland through multiple mechanisms. Interestingly, both fibroblast activation and EMT

have been indicated to be induced by TGF- β signalling and oxidative stress [30, 35, 36]. IR is known to cause the overproduction of TGF- β and reactive oxygen species, which exert oxidative stress, but further research is necessary to confirm their involvement in the pathophysiology of RIHT [37, 38]. Unlike the changes observed in ECM protein expression, few quantitative changes were observed regarding expression of thyroid-specific proteins following IR. A continuous upregulation of NKX2-1 was shown by IF imaging. Although heterozygous mutations or haploinsufficiency of NKX2-1 are associated with brain-lung-thyroid syndrome which incorporates primary hypothyroidism, little research is available where upregulation of NKX2-1 has been observed in the thyroid [39]. NKX2-1 expression has been widely studied during lung disease, and has been associated with inflammation as well as disease progression but even here results are not yet fully understood [40]. Theoretically, as NKX2-1 is a transcription factor for multiple thyroid-specific proteins, including Tg, TPO and TSHR, an increase in NKX2-1 expression should coincide with an increase of the aforementioned proteins [41, 42]. However, this result was not observed here. While an increase in Tg production may have occurred throughout the study, the localizational change of Tg following IR from the follicular lumen to the thyrocyte cytoplasm suggests that either follicles may be unable to store this protein or thyrocytes are prevented from releasing it. These possibilities could be explained by the observed loss of cell polarity and ECM proteins, specifically ECAD and ZO-1 which are essential in maintaining the thyroid follicular membrane. Nevertheless, the prevention of Tg storage alone would be sufficient to reduce thyroid hormone production, and hence lead to the development of RIHT. Following the observation of both ECM and thyroid-related protein expression changes in the thyroid gland after IR exposure, it is evident that maintenance of their expressional levels is essential in preventing the onset of RIHT. As such, these proteins may provide a valuable tool to assess novel tissue engineered therapeutics for RIHT. To evaluate the potential of the above discussed biomarkers for the assessment of tissue engineered RIHT therapeutics *in vitro*, an electrospun PCL scaffold, displaying consistent characteristics, was fabricated and seeded with non-cancerous human thyroid Nthy-ori 3-1 cells. The scaffolds were able to support good proliferation and metabolic activity of Nthy-ori 3-1 cells. According to IF imaging, Nthy-ori 3-1 cells cultured on the scaffolds displayed steadily increasing levels of NKX2-1, TPO, Tg and TSHR. This indicates that the scaffold may be able to support thyroid hormone production, as previous studies suggest little difference between the response of Nthy-ori 3-1 and primary thyroid cells cultured on scaffolds with comparable properties [8]. Interestingly, a transient decrease in ZO-1 and increase in COL1 as well as α SMA was observed in Nthy-ori 3-1 cells cultured on the scaffold, mimicking the long-term effects of IR on thyroid tissue. It has previously been reported that trypsinisation of cells can decrease cellular levels of reduced

glutathione, a protein able to mitigate cellular oxidative stress [43, 44]. Furthermore, little changes have been found in thyroid-related functional protein expression of Nthy-ori 3-1 cells, including that of TPO, when exposed to oxidative stress [45]. This, taken together with the phenomenon of ‘culture shock’ which cells can experience when being cultured in a foreign environment, such as an electrospun scaffold, may provide an explanation for these ECM-related protein expression changes. Nevertheless, by the end of study expression of ZO-1 and COL1 returned to levels comparable to initial observation. The increase seen in expression of α SMA in this context may indicated that the scaffold may mechanically or topographically promote a contractile cytoskeleton, reflecting improved tissue mimicry [46, 47]. The increase in ECAD, which is essential for homophilic cell-cell adhesion of thyrocyte, noted during culture of Nthy-ori 3-1 cells is most likely due to an increased number of cells on the scaffold over time, and indicates improved cell adhesion capabilities [48, 49]. This is further evidenced by the increasing appearance of cell-surface proteins and cell-cell junctions in the SEM images of Nthy-ori 3-1 cells cultured on the scaffold. Despite these promising results, there are still some considerations which need to be taken into account. Firstly, while the murine model used to assess expressional changes which may contribute to RIHT shows an evident decrease in T4, it was originally developed for investigating xerostomia as a result of radiotherapy, and as such, the model may not ideally represent RIHT in terms of dose or regime. Moreover, the quantitative analysis of protein expression undertaken from IF images provide a relative abundance to enable comparison between samples and not an absolute quantity. As certain polymers such as PCL are known to diffract visible light, localization of proteins in cell-seeded scaffolds cannot be accurately determined through IF imaging alone [50]. Lastly, although unlikely as evidenced by previous research, primary thyrocytes may provide a distinct response to culture on scaffolds which is not recapitulated in immortalized thyroid cells [8]. Future research directions should delve deeper into elucidating the molecular mechanisms underlying the response of thyroid tissue to IR. Investigating the signalling pathways regulating fibrosis and thyroid-specific protein expression could provide valuable insight into the pathophysiology of RIHT and inform the development of targeted therapeutics. Additionally, uncovering these mechanisms may aid in optimization of tissue-engineered scaffolds through the incorporation of bioactive molecules [51, 52]. By modulating cellular responses, the local immune microenvironment and tissue regeneration, bioactive scaffold modifications present a promising avenue for enhancing therapeutic efficacy of targeted RIHT treatments.

4 Conclusion

In summary, this research unveiled significant alterations in expression of essential ECM-related proteins and thyroid-related functional proteins following IR using an *in vivo* model, shedding light on potential biomarkers for evaluating scaffold-mediated thyroid function *in vitro*. The findings reveal the complexity of RIHT pathophysiology and the multifaceted response of thyroid tissue to both IR and scaffold intervention. By elucidating the dynamic interplay of proteins involved in fibrotic processes following IR and thyroid-specific function, this research provides insight into targeted therapeutic approaches for RIHT management. Moreover, this study underscores the promise of tissue-engineered scaffolds as a viable avenue for RIHT treatment, supported by their ability to sustain thyroid cell proliferation and metabolic activity while modulating protein expression over time. These findings contribute to the advancement of our understanding of RIHT pathogenesis and offer potential avenues for developing effective tissue engineering interventions for thyroid dysfunction post-IR.

5 Experimental Section

5.1 Mice

All procedures adhered to the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and were approved by the U.K. Home Office, performed by the project license holder PB5FC9BD2 (Emmerson). Male C57BL/6J mice aged 8-10 weeks of age were used. All mice were randomized into experimental groups; and the researcher undertaking imaging and subsequent analysis was blinded to experimental conditions.

5.2 Induction of RIHT

Prior to g-irradiation (IR), mice were anesthetized using 1mg/kg medetomidine hydrochloride (Dormitor) and 75mg/kg ketamine (Ketavet) in 0.9% saline (Thermo Fisher Scientific) by IP injection. IR of mice was conducted using a single ^{137}Cs source (10Gy) in a Shepherd Mark-I-68A irradiator (JL Shepherd & Associates) with only the neck exposed and the head and body shielded by lead, as previously described (**Figure1b**) [15]. Following 20mins of anaesthesia, mice were administered 1mg/kg atipamezole (Antisedan; reversal agent) and allowed to recover in a heated cabinet before being returned to normal housing. Mice were subsequently provided with a soft diet and DietGel (Clear H_2O) *ad libitum* to mitigate the effects of

head and neck radiation injury. Mice were euthanized at day 3 or 28 post-IR (**Figure1b**) via CO₂ inhalation and confirmed by cervical dislocation. Induction of RIHT was confirmed via the lack of thyroxine (T4) by immunofluorescent analysis. Non-IR control mice were not anesthetized as a refinement, since published research has demonstrated no difference between control anesthetized and control non-anesthetized animals [14, 15].

5.3 Tissue processing for histology

Thyroid glands were resected and fixed for 4-6 hours in 4% paraformaldehyde (PFA, Thermo Fisher Scientific) at room temperature (RT) with constant agitation. This was followed by PBS washes (3x10min, Merck) and incubation in 30% sucrose before embedding in optimal cutting temperature compound (OCT, Leica). 10µm sections were cut using a cryostat (Leica) and stored at -20°C prior to analysis.

5.4 Immunofluorescent Analysis

Immunofluorescent analysis of tissue sections (N=8 per group) and cell-seeded scaffolds (N=5) followed a previously described protocol. Briefly, fixed tissue sections or scaffolds were permeabilized with 0.1% Triton for 10min followed by PBS washes (3x10min, Merck). Samples were blocked for 2h with 5% donkey serum, 5% bovine albumin serum (BSA, Sigma-Aldrich) in 0.01% PBS-Tween-20 (PBST, Sigma-Aldrich) at RT. For tissue samples, mouse-on-mouse IgG blocking reagent (Vector Laboratories) was added to negate non-specific binding. Samples were incubated with primary antibodies overnight at 4°C in 0.01% PBST (Online Resource 1), followed by PBST washes (3x10min). Antibodies were detected using Cy3-, AlexaFluor® 488- and Alexa Fluor® 647-conjugated secondary Fab fragment antibodies (1:300; Jackson ImmunoResearch) and nuclei were stained using Hoechst 33342 (1:1000, Sigma-Aldrich) in 0.01% PBST for 2hr at RT, while protected from light. Tissue sections were mounted using Prolong Gold anti-fade mounting medium (Invitrogen) and scaffolds using 0.01% PBST. Images of tissue samples and scaffolds were acquired on a Leica SP8 4D confocal microscope and a Nikon Ti-E inverted widefield microscope, respectively. Fluorescent images were segmented using CellPose2.0 to identify regions of interest (ROIs). The relative mean fluorescent intensity (MFI) of ROIs was calculated and background fluorescence subtracted in ImageJ.

5.5 Electrospinning

10% (w/v) PCL (Mn = 80,000 Da, Sigma Aldrich) was dissolved in Hexafluoroisopropanol (HFIP)

(Manchester Organics). The PCL concentration and electrospinning parameters were based on previous research [10]. Electrospinning was performed using a syringe pump EP-H11 (Harvard Apparatus) and an EC-DIG electrospinning system (IME technologies) via a 24G needle, with a flow rate of 1ml/hr, a positive voltage of 16kV and a negative voltage of -4kV. Electrospun fibres were collected on non-stick aluminium foil, on a rotating mandrel (250rpm) placed 15cm away from the needle and allowed to dry overnight.

5.6 Scaffold Characterization

To ensure the scaffold displayed physical and mechanical features consistent with the literature, the following characteristic assessments were conducted:

Scaffold morphology and topography were visualized using an emission scanning electron microscope (SEM, HitachiS4700) with a 5 kV accelerating voltage and a 12 mm working distance, following sputter coating with gold-palladium (60:40, Emscope SC500A). The fibre diameter was calculated as an average (N=50) of individual fibres from 5 regions of interest (ROI) measure in ImageJ.

Tensile properties of 25x5mm scaffold (N=5) were measured with an Instron 3367 (50N load cell, Instron) through stretch until failure at a rate of 5mm/min. Young's Modulus, E , was calculated according to **Equation 1**, where σ denotes stress, ε denotes strain, F is force, A is scaffold cross-sectional area, L_0 is the initial length and ΔL is change in length, as previously described [12].

$$E = \frac{\sigma}{\varepsilon} = \frac{FL_0}{A\Delta L} \quad (1)$$

To investigate porosity, scaffolds (N=5) were visualized with a DMK41AU02 monochrome camera (5Hz). Scaffold thickness was measured in ImageJ and porosity calculated via **Equation 2**, assuming a polymer density of 1.145g/cm³ at 25°C according to the manufacturer [53].

$$\text{Porosity} = 1 - \frac{\rho_{\text{measured}}}{\rho_{\text{theoretical}}} \times 100 \quad (2)$$

Scaffold hydrophilicity (N=5) was calculated based on the angle of a 5µl drop of water at 0.2 seconds using the ImageJ LBADSA plugin on pictures obtained with a DMK41AU02 monochrome camera (5Hz).

Lastly, crystallinity and melting temperature of 5mg scaffold (N=3) was analysed via subjection to two thermal cycles from 5-75°C, at a rate of 10°C/min, using a differential scanning calorimeter (DSC 8000, PerkinElmer) with a nitrogen atmosphere (20mL/min flow rate). Calculations were undertaken as previously described [54].

The scaffold characterization is displayed in Online Resource 2.

5.7 Scaffold Seeding & Culture

10mm scaffold biopsy punches (Peddinghaus) were sterilized in 70% ethanol for 12h. Scaffolds were incubated for 24h with 1% Antibiotic-Antimycotic in PBS (5% CO₂, 37C) followed by 6h in complete media. Nthy-ori 3-1 cells, obtained at passage 0 from the European Collection of Authenticated Cell Cultures (ECACC), were expanded to passage 4 before use. Cells were cultured with RPMI-1640 medium (10% Foetal Bovine Serum, 2mM L-Glutamine, 1% Anti-Anti) (Sigma Aldrich) as per manufacturer's directions (5% CO₂, 37C). Nthy-ori 3-1 cells were lifted at 80% confluency and drip seeded at 10,000 cells/scaffold. In brief, 100µl of cell suspension was seeded onto the scaffolds; following 2h incubation (5% CO₂, 37°C), an additional 400µl of complete medium was added, changed after 24h and every 48h subsequently. Seeded scaffolds were assessed at 24h, 7 days and 14 days.

5.8 Metabolic Activity

The CellTitreBlue (CTB, Promega) assay was performed as previously described. Briefly, viable cells are convert the dye resazurin to fluorescent resorufin, whilst nonviable cells cannot complete this process due to a loss of metabolic activity [55]. Scaffolds were transferred to a new plate and 500µl of media-CTB (ratio 4:1) was added, followed by 3h incubation (37°C, 5% CO₂) protected from light. Fluorescent intensity of 100µl solution was measured in a black well plate with a Modulus II microplate reader (N=6) at an excitation wavelength of 525nm and emission wavelength of 580-640nm. All data has been represented with removed background fluorescence.

5.9 DNA Quantification

Scaffolds were freeze-dried for 12h before a 12h enzymatic digestion (2.5 units papain/ml, 5mM Cysteine, 5mM ethylenediaminetetraacetic (EDTA) in DNase-free water) at 65°C with agitation. The Quant-IT PicoGreen® dsDNA assay (Life Technologies™) was performed to quantify the DNA content, as according to manufacturer's instructions. Fluorescent intensity measurements were read in a Modulus™ II microplate reader (N=6) at an excitation wavelength of 480 nm and emission wavelength of 510–570 nm. A standard lambda dsDNA curve of graded known concentrations was used to calibrate fluorescence intensity versus dsDNA concentration.

5.10 Osmium Staining

Scaffolds were washed with PBS (3x10min) and fixed with 4% Glutaraldehyde for 12h before storage at 4°C in PBS. 2% Osmium in deionized water was applied to the scaffolds for 30min, followed by 4x5min washes with deionized water. Samples were dehydrated with sequential ethanol washes (30-100%) and hexamethyldisilazane (HMDS) before storage with light protection. Scaffolds were sputter coated prior to imaging (Emscope SC500A) using gold-palladium (ratio 60:40). Cell and scaffold interactions were studied with a JEOL JSM-IT100 scanning electron microscope and software ImageJ.

5.11 Statistical Analysis

Data was expressed as mean $\pm$ standard deviation. A Shapiro-Wilk test was applied to ensure normal distribution, with * $p > 0.05$ indicating that data was normally distributed. An F-test was applied to determine equality of variance, with * $p > 0.05$ indicating no significant difference in variance within the dataset. Significant results were determined using a one-way ANOVA with a *post-hoc* Tukey test, where * $p < 0.05$, ** $p < 0.1$ and *** $p < 0.001$. Statistical analysis was conducted using RStudio1.4.

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Author Contributions: MH performed the experimental procedures, drafted the manuscript and tables, and edited the manuscript. TF and DG aided in conducting *in vivo* experimentation, data analysis and hypothesis generation. EE and AC contributed to hypothesis generation, funding acquisition and edited the manuscript.

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Data Availability Statement The data that support the findings of this study are available from the corresponding author upon reasonable request.

References

- [1] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, A. Jemal, *CA: A Cancer Journal for Clinicians* **2018**, *68*, 6 394.
- [2] Cancer Research UK, Breast cancer statistics — Cancer Research UK, **2025**, URL <https://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/breast-cancer#heading-Zero>.
- [3] L. Zhou, J. Chen, C.-J. Tao, M. Chen, Z.-H. Yu, Y.-Y. Chen, *Journal of Cancer* **2021**, *12* 451.
- [4] M. Khalifa, O. Abbas, M. Soliman, W. Arafat, *International Journal of Radiation Oncology*Biology*Physics* **2023**, *117*, 2 e185.
- [5] J. Gaur, P. Verma, A. Arya, L. Mohan, *International Journal of Academic Medicine and Pharmacy* **2023**.
- [6] M. Bakhshandeh, B. Hashemi, S. R. M. Mahdavi, A. Nikoofar, M. Vasheghani, A. Kazemnejad, *International Journal of Radiation Oncology Biology Physics* **2013**, *85*, 2 514.
- [7] M. Heim, I. J. Nixon, E. Emmerson, A. Callanan, *Frontiers in Endocrinology* **2022**, *13*.
- [8] M. Heim, E. L. Handley, E. Emmerson, A. Callanan, *Materialia* **2024**, *36* 102115.
- [9] G. G. Flores-Rojas, B. Gómez-Lazaro, F. López-Saucedo, R. Vera-Graziano, E. Bucio, E. Mendizábal, *Macromolecular Materials and Engineering* **2023**, *3*, 3 524.
- [10] G. Kathiresan, K. Adaikalam, H. S. Kim, *Journal of Applied Polymer Science* **2025**, *142*, 33 e57309.
- [11] Y. Gao, T. S. Bate, A. Callanan, *ACS Applied Bio Materials* **2023**, *6*, 6 2158.
- [12] J. B. Qu, G. F. Li, Y. J. Li, Y. J. Sun, X. Ma, H. J. Che, Y. Li, X. Y. Sui, X. Wang, *Macromolecular Materials and Engineering* **2022**, *307*, 7 2100961.
- [13] Culture Collections, FRTL-5, **2025**, URL <https://www.culturecollections.org.uk/nop/product/frtl-5>.
- [14] E. Emmerson, A. J. May, L. Berthoin, N. Cruz-Pacheco, S. Nathan, A. J. Mattingly, J. L. Chang, W. R. Ryan, A. D. Tward, S. M. Knox, *EMBO Molecular Medicine* **2018**, *10*.

- [15] J. G. McKendrick, G. R. Jones, S. S. Elder, E. Watson, W. T'Jonck, E. Mercer, M. S. Magalhaes, C. Rocchi, L. M. Hegarty, A. L. Johnson, C. Schneider, B. Becher, C. Pridans, N. Mabbott, Z. Liu, F. Ginhoux, M. Bajenoff, R. Gentek, C. C. Bain, E. Emmerson, *Science Immunology* **2023**, 8.
- [16] S. Banerjee, Q. Fu, S. K. Shah, S. B. Melnyk, E. Sterneck, M. Hauer-Jensen, S. A. Pawar, *Scientific Reports* 2019 9:1 **2019**, 9, 1 13953.
- [17] D. V. Krysko, T. Vanden Berghe, K. D'Herde, P. Vandenabeele, *Methods (San Diego, Calif.)* **2008**, 44, 3 205.
- [18] C. T. Eckert, J. G. Probst, S. Galinson, *Radiology* **1937**, 29, 1 40.
- [19] L. J. DeGroot, *Baillière's Clinical Endocrinology and Metabolism* **1988**, 2, 3 777.
- [20] Z. Lin, L. Chen, Y. Fang, A. Cai, T. Zhang, V. W. Wu, *Head & neck* **2014**, 36, 2 171.
- [21] T. A. Wynn, *The Journal of pathology* **2008**, 214, 2 199.
- [22] T. N. Wight, S. Potter-Perigo, *American journal of physiology. Gastrointestinal and liver physiology* **2011**, 301, 6.
- [23] J. Herrera, C. A. Henke, P. B. Bitterman, *The Journal of clinical investigation* **2018**, 128, 1 45.
- [24] M. W. Parker, D. Rossi, M. Peterson, K. Smith, K. Sikstrom, E. S. White, J. E. Connett, C. A. Henke, O. Larsson, P. B. Bitterman, *The Journal of clinical investigation* **2014**, 124, 4 1622.
- [25] J. M. Straub, J. New, C. D. Hamilton, C. Lominska, Y. Shnayder, S. M. Thomas, *Journal of Cancer Research and Clinical Oncology* **2015**, 141 1985.
- [26] S. Ueha, F. H. Shand, K. Matsushima, *Frontiers in immunology* **2012**, 3, APR.
- [27] E. E. Torr, C. R. Ngam, K. Bernau, B. Tomasini-Johansson, B. Acton, N. Sandbo, *Journal of Biological Chemistry* **2015**, 290, 11 6951.
- [28] T. Ebara, N. Mitsuhashi, Y. Saito, T. Akimoto, H. Niibe, *International Journal of Radiation Oncology Biology Physics* **1998**, 41, 3 669.
- [29] S. Lovisa, *Frontiers in pharmacology* **2021**, 12.
- [30] E. Sari, F. Oztay, A. E. Tasci, *Journal of Steroid Biochemistry and Molecular Biology* **2020**, 202.

- [31] R. K. Kim, N. Kaushik, Y. Suh, K. C. Yoo, Y. H. Cui, M. J. Kim, H. J. Lee, I. G. Kim, S. J. Lee, *Oncotarget* **2016**, 7 53430.
- [32] S. Zhou, M. Zhang, C. Zhou, W. Wang, H. Yang, W. Ye, *Critical Reviews in Oncology/Hematology* **2020**, 150.
- [33] D. Kyuno, A. Takasawa, S. Kikuchi, I. Takemasa, M. Osanai, T. Kojima, *Biochimica et Biophysica Acta - Biomembranes* **2021**, 1863, 3.
- [34] D. Neyrinck-Leglantier, J. Lesage, S. Blacher, A. Bonnomet, W. Hunziker, A. Noël, V. Dormoy, B. Nawrocki-Raby, C. Gilles, M. Polette, *Frontiers in cell and developmental biology* **2021**, 9.
- [35] A. Vallée, Y. Lecarpentier, *Cell & Bioscience 2019 9:1* **2019**, 9, 1 98.
- [36] E. Giannoni, M. Parri, P. Chiarugi, *Antioxidants & redox signaling* **2012**, 16, 11 1248.
- [37] B. Farhood, E. khodamoradi, M. Hoseini-Ghahfarokhi, E. Motevaseli, H. Mirtavoos-Mahyari, A. Eleojo Musa, M. Najafi, *Pharmacological Research* **2020**, 155 104745.
- [38] Z. Zheng, J. Su, X. Bao, H. Wang, C. Bian, Q. Zhao, X. Jiang, *Frontiers in immunology* **2023**, 14.
- [39] A. Thorwarth, S. H. Sarah, P. Schrumpf, I. Müller, S. Jyrch, C. Dame, H. Biebertmann, G. Kleinau, J. Katchanov, M. Schuelke, G. Ebert, A. Steininger, C. Bönnemann, K. Brockmann, H. J. Christen, P. Crock, F. deZegher, M. Griesse, J. Hewitt, S. Ivarsson, C. Hübner, K. Kapelari, B. Plecko, D. Rating, I. Stoeva, H. H. Ropers, A. Grütters, R. Ullmann, H. Krude, *Journal of Medical Genetics* **2014**, 51, 6 375.
- [40] L. Yang, M. Lin, W. J. Ruan, L. L. Dong, E. G. Chen, X. H. Wu, K. J. Ying, *Journal of Zhejiang University. Science. B* **2012**, 13, 11 855.
- [41] D. Civitareale, R. Lonigro, A. J. Sinclair, R. Di Lauro, *The EMBO journal* **1989**, 8, 9 2537.
- [42] D. Civitareale, M. P. Castelli, P. Falasca, A. Saiardi, *Molecular endocrinology (Baltimore, Md.)* **1993**, 7, 12 1589.
- [43] H. L. Huang, H. W. Hsing, T. C. Lai, Y. W. Chen, T. R. Lee, H. T. Chan, P. C. Lyu, C. L. Wu, Y. C. Lu, S. T. Lin, C. W. Lin, C. H. Lai, H. T. Chang, H. C. Chou, H. L. Chan, *Journal of Biomedical Science 2010 17:1* **2010**, 17, 1 36.

- [44] F. Nuhu, A. Gordon, R. Sturmey, A. M. Seymour, S. Bhandari, *Molecules (Basel, Switzerland)* **2020**, *25*, 18.
- [45] J. Zhuang, Z. Jiang, D. Chen, J. Li, M. J. C. Crabbe, M. Qiu, Y. Zheng, W. Qu, *Environmental Science & Technology* **2023**, *57*, 15 6072.
- [46] B. Hinz, G. Celetta, J. J. Tomasek, G. Gabbiani, C. Chaponnier, <https://doi.org/10.1091/mbc.12.9.2730> **2017**, *12*, 9 2730.
- [47] Y. Han, Z. Shao, Y. Zhang, H. Zhao, Z. Sun, C. Yang, H. Tang, Y. Han, C. Gao, *Scientific Reports* *2024 14:1* **2024**, *14*, 1 17015.
- [48] L. Li, S. A. Bennett, L. Wang, *Cell adhesion & migration* **2012**, *6*, 1 59.
- [49] G. Calì, M. Zannini, P. Rubini, C. Tacchetti, B. D’Andrea, A. Affuso, T. Wintermantel, O. Boussadia, D. Terracciano, D. Silberschmidt, E. Amendola, M. De Felice, G. Schütz, R. Kemler, R. Di Lauro, L. Nitsch, *Endocrinology* **2007**, *148*, 6 2737.
- [50] J. M. Weissman, H. B. Sunkara, A. S. Tse, S. A. Asher, *Science* **1996**, *274*, 5289 959.
- [51] B. Baskapan, A. Callanan, *Tissue Engineering and Regenerative Medicine* **2022**, *19* 73.
- [52] E. Antmen, N. E. Vrana, V. Hasirci, *Biomaterials science* **2021**, *9*, 24 8090.
- [53] L. Westwood, E. Emmerson, A. Callanan, *Frontiers in Bioengineering and Biotechnology* **2023**, *11*.
- [54] E. L. Handley, A. Callanan, *Journal of Applied Polymer Science* **2023**, *140*.
- [55] J. Wesierska-Gaadek, M. Gueorguieva, C. Ranftler, G. Zerza-Schnitzhofer, *Journal of cellular biochemistry* **2005**, *96*, 1 1.

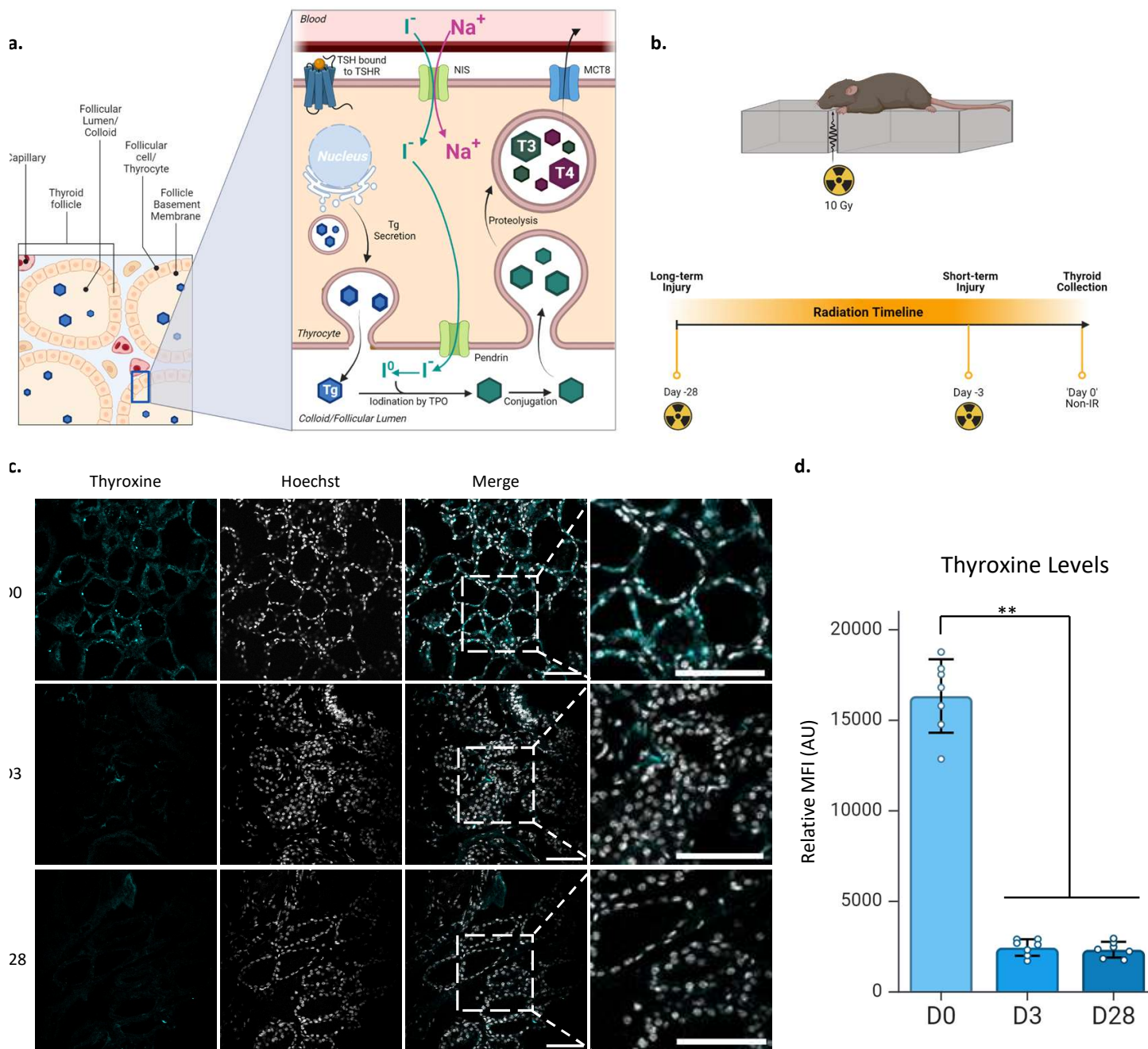


Figure 1: Induction of RIHT a. Overview of the thyroid microanatomy and hormone synthesis process. b. Schematic of the experimental in vivo radiation schedule c. Representative images of murine thyroid glands stained for thyroxine (T4) pre-irradiation (D0) and day 3 and 28 post-irradiation. Scale bar=60 μ m d. Relative levels of thyroxine expression in the thyroids pre- and post-irradiation. Data represented as mean $\pm$ SD, N=7, Statistics performed: one-way ANOVA and Tukey post hoc test, **=p-value<0.01. Created with Biorender

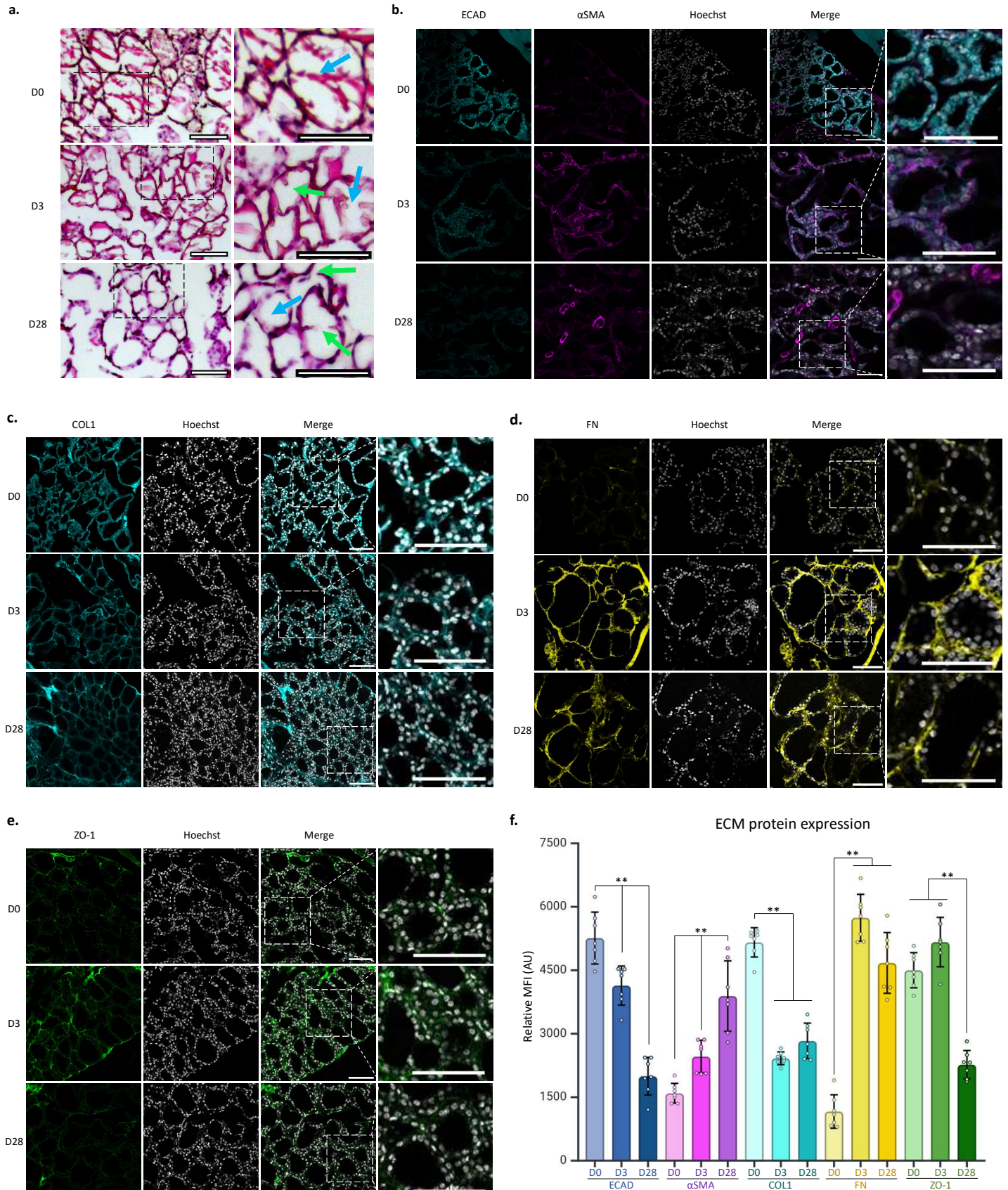


Figure 2: Expressional changes of thyroid ECM proteins following irradiation: Images display thyroids pre- and post-irradiation fluorescently stained for a. H&E images with green arrows indicating breakdown of follicle basement membranes and loss of cell polarity, and blue arrows indicating the colloid and lack thereof. b. E-Cadherin (ECAD, cyan) and alpha-smooth-muscle actin (α SMA, magenta) c. Collagen-1 (COL1, cyan) d. fibronectin (FN, yellow) and e. zonula occludens-1 (ZO-1, green) f. Relative levels of ECM protein expression in the thyroids pre- and post-irradiation. Data represented as mean $\pm$ SD, N=7, scale bar = 60 μ m, Statistics performed: one-way ANOVA and Tukey post hoc test, **=p-value<0.01

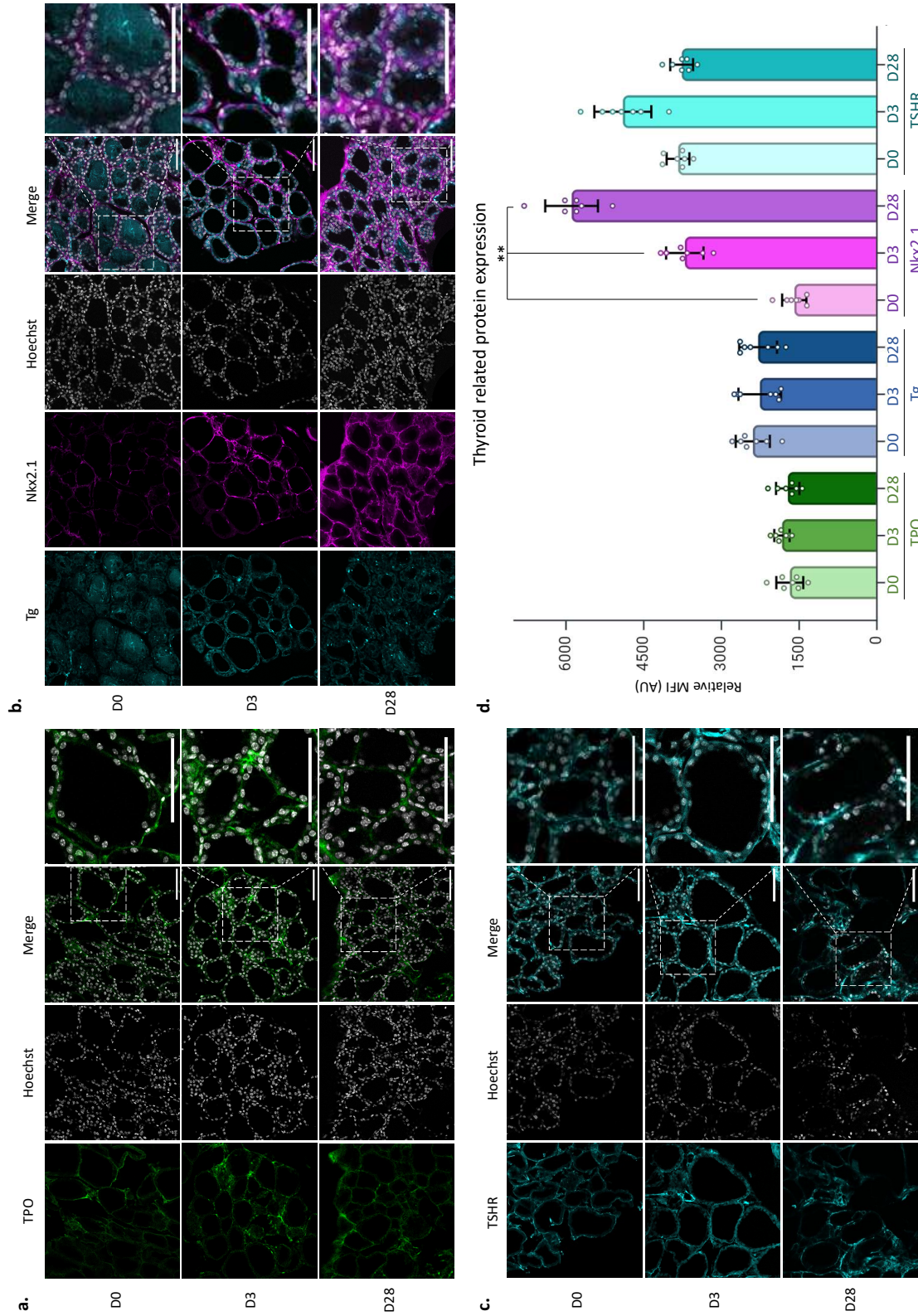


Figure 3: Culture of Nthy-ori 3-1 cells on electrospun scaffolds: a. Schematic of the production and experimental timeline of electrospun scaffolds. Figure created with Biorender. PCL= polycaprolactone, HFIP=hexafluoroisopropanol q=electric charge b. Metabolic activity of Nthy-ori 3-1 cells seeded onto electrospun scaffolds c. Proliferative activity of Nthy-ori 3-1 cells seeded on scaffolds as indicated by the DNA content. Data represented as mean $\pm$ SD, N=6, Statistics performed: one-way ANOVA and Tukey post hoc test, **=p-value $\leq$ 0.01, *=p-value $\leq$ 0.05

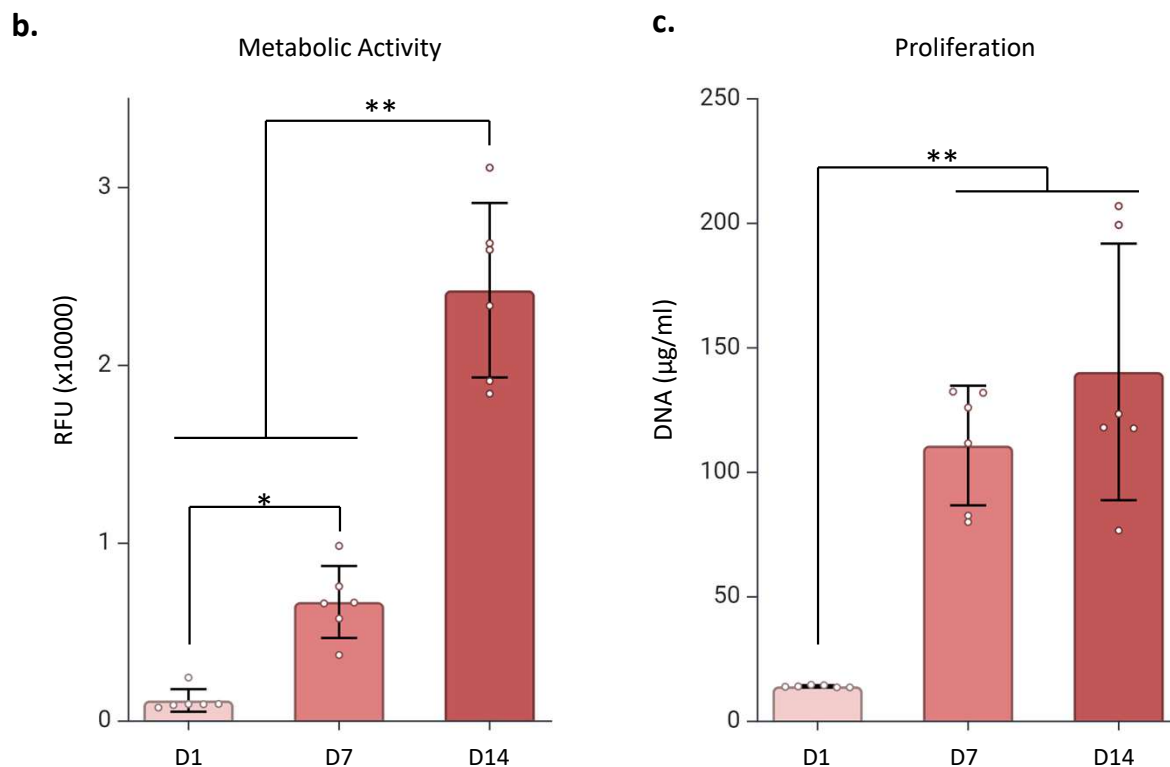
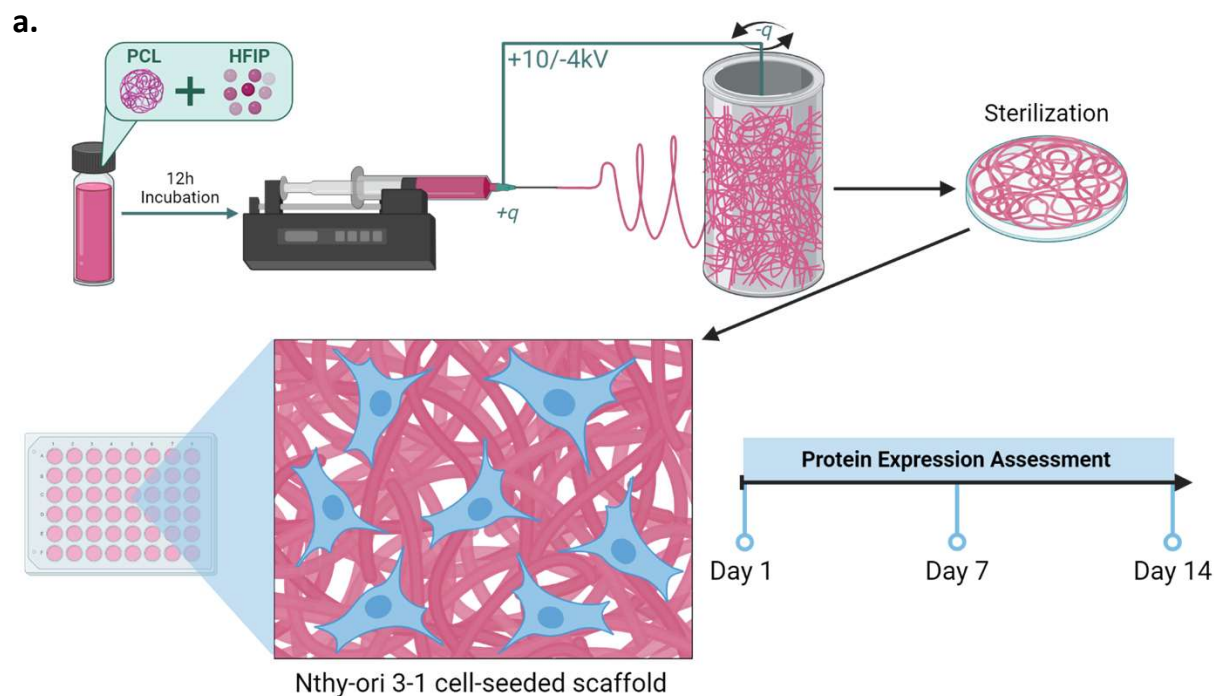


Figure 4: Expressional changes of thyroid-related proteins following irradiation: Images display thyroids pre- and post-irradiation fluorescently stained for a. thyroid peroxidase (TPO, green) b. thyroglobulin (Tg, cyan) and NK2 homebox (Nkx2-1, magenta) c. thyroid-stimulating-hormone receptor (TSHR, cyan) d. Relative levels of thyroid-related protein expression in the thyroids pre- and post-irradiation. Data represented as mean $\pm$ SD, N=7, scale bar= 60µm, Statistics performed: one-way ANOVA and Tukey post hoc test, **=p-value<0.01

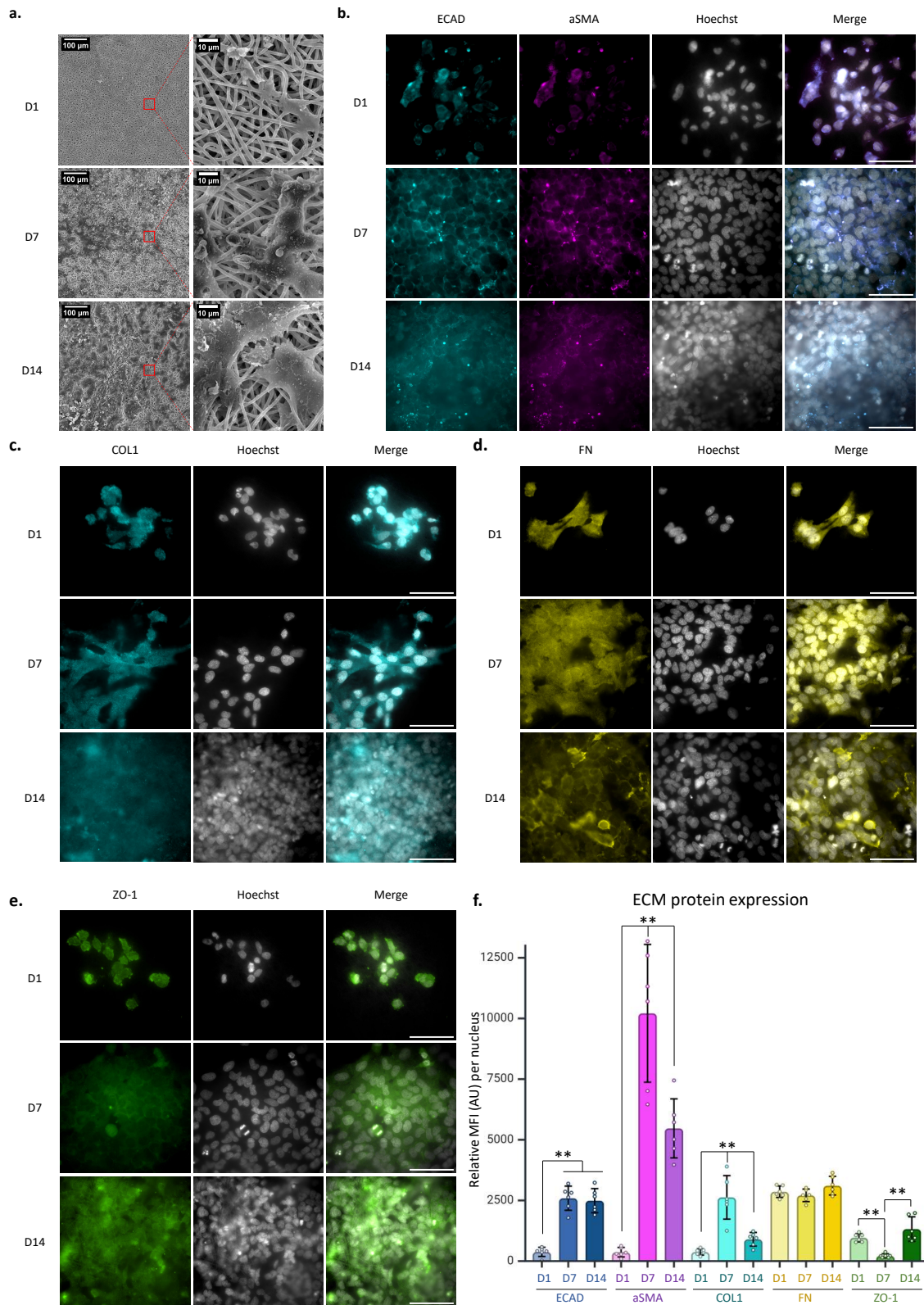


Figure 5: Expressional changes of thyroid ECM proteins on electrospun scaffolds: a. SEM images of osmium stained Nthy-ori 3-1 cell-seeded scaffolds at x150 magnification (left) and x1500 magnification (right). Images display Nthy-ori 3-1 cell-seeded scaffolds over a 14-day period stained for b. E-Cadherin (ECAD, cyan) and alpha-smooth-muscle actin (aSMA, magenta) c. collagen-1 (COL1, cyan) d. fibronectin (FN, yellow) and e. zonula occludens-1 (ZO-1, green) f. Relative levels of ECM protein expression normalized to nuclei count. Data represented as mean $\pm$ SD, n = 6, Scale bar = 20 μ m Statistics performed: one-way ANOVA and Tukey post hoc test, **=p-value<0.01

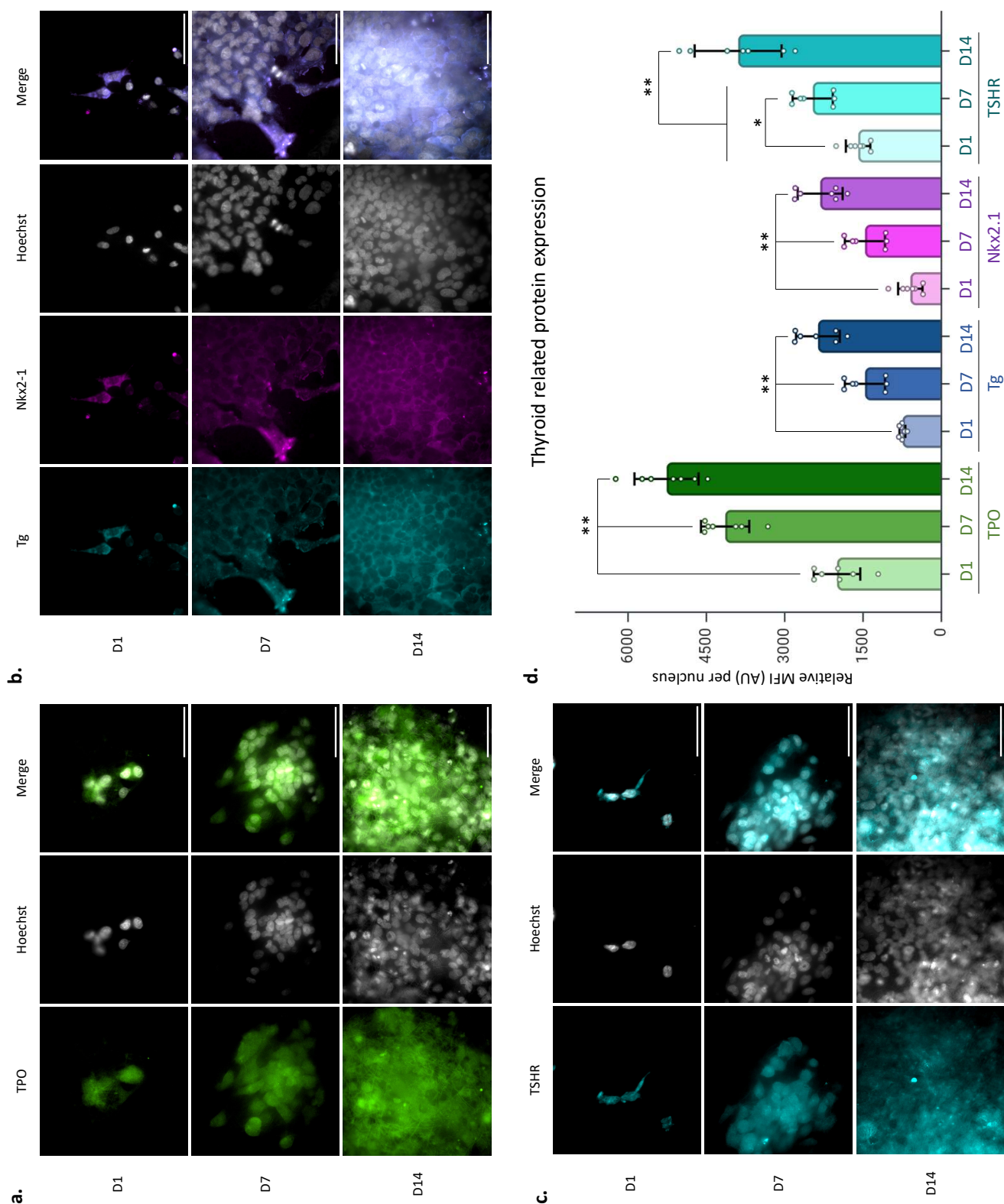


Figure 6: Expressional changes of thyroid-related proteins on electrospun scaffolds: Images display Nthy-ori 3-1 cell-seeded scaffolds fluorescently stained for a. thyroid peroxidase (TPO, green) b. thyroglobulin (Tg, cyan) and NK2 homebox (Nkx2-1, magenta) c. thyroid-stimulating-hormone receptor (TSHR, cyan) d. Relative levels of thyroid-related protein expression in the thyroids pre- and post-irradiation. Data represented as mean $\pm$ SD, N=6, scale bar= 20 μ m, Statistics performed: one-way ANOVA and Tukey post hoc test, *= p -value $\leq$ 0.05, **= p -value $<$ 0.01

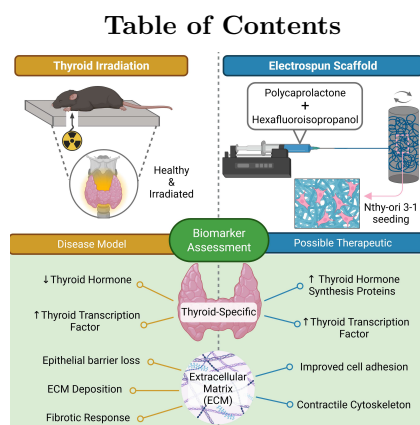


Table of Contents: Radiation-induced hypothyroidism disrupts thyroid architecture, reducing hormone production and altering ECM and junctional protein expression. This study identifies key biomarkers of fibrosis and epithelial dysfunction in vivo, then validates their relevance using thyroid cells cultured on electrospun PCL scaffolds. Scaffold-supported cells regain thyroid-specific functions, demonstrating the platform's potential for evaluating biomimetic materials and guiding RIHT tissue-engineering strategies.

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