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Effect of scaffold properties on adhesion and maintenance of boundary cap neural crest stem cells in vitro

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Abstract

Optimal combination of stem cells and biocompatible support material is a promising strategy for successful tissue engineering. The required differentiation of stem cells is crucial for functionality of engineered tissues and can be regulated by chemical and physical cues. Here we examined how boundary cap neural crest stem cells (bNCSCs) are affected when cultured in the same medium, but on collagen- or laminin-polyacrylamide (PAA) scaffolds of different stiffness (0.5, 1, or ~7 kPa). bNCSCs displayed marked differences in their ability to attach, maintain a large cell population and differentiate, depending on scaffold stiffness. These findings show that the design of physical cues is an important parameter to achieve optimal stem cell properties for tissue repair and engineering.

KEYWORDS

differentiation, neural crest stem cell, polyacrylamide, scaffold, survival

1 | INTRODUCTION

Stem cells are attractive for regenerative medicine as a source for cell replacement therapy, as well as for their potential to modify disease processes and promote tissue repair. The interplay between stem cells and their extracellular environment plays a crucial role in their survival, differentiation, and functionality (Burdick & Vunjak-Novakovic, 2009). This interplay occurs mainly through molecular interactions, but mechanical conditions such as extracellular matrix surface properties also play an important role. Understanding how chemical and physical cues cooperate in neural stem cell differentiation is therefore critical for optimal application of stem cell-based therapy.

We have previously explored the properties of boundary cap neural crest stem cells (bNCSCs), a transient neural crest-derived group of cells (Aldskogius et al., 2009; Hjerling-Leffler et al., 2005), which are able to differentiate into neurons and glia in vitro and after transplantation to the peripheral (Aquino et al., 2006) or central nervous system (Radomska & Topilko, 2017; Trolle, König, Abrahamsson, Vasylovska, & Kozlova, 2014; Zujovic et al., 2010, 2011) in vivo.

In addition to their robust survival and broad differentiation potential, they display a remarkable ability to promote survival and support the function of different types of co-cultured cells. Thus, bNCSCs stimulate proliferation of co-cultured (Grouwels et al., 2012; Ngamjariyawat, Turpaev, Vasylovska, Kozlova, & Welsh, 2013; Ngamjariyawat, Turpaev, Welsh, & Kozlova, 2012; Wang et al., 2016) and co-implanted insulin-producing pancreatic beta-cells (Lau, Vasylovska, Kozlova, & Carlsson, 2015; Olerud et al., 2009). Co-transplantation of bNCSCs and human pancreatic islets increased islet vascularization and innervation, most likely mediated by diffusible factors released from the bNCSCs (Grapensparr et al., 2015; Lau et al., 2015).

bNCSCs also promote survival of excitotoxically challenged spinal cord neurons in vitro (Schizas et al., 2018), showing that their beneficial effects are not limited to pancreatic beta-cells. bNCSC-derived astrocytes were able to protect motor neurons with a mutation associated with amyotrophic lateral sclerosis (ALS) from oxidative stress to a degree surpassing that of embryonic stem cell-derived astrocytes (Aggarwal, Hoeber, Ivert, Vasylovska, & Kozlova, 2017). At the same time, bNCSC-derived astrocytes have shown extreme resistance to

oxidative stress (Aggarwal et al., 2017), and other environmental challenges (own, unpublished data). Recently, injection of bNCSCs into the spinal cord ventral horn of an ALS mouse model was shown to delay disease progression (Leyton-Jaimes et al, in preparation for publication).

It was previously demonstrated that neural stem cell differentiation depends on the stiffness of the scaffolds. Thus oligodendrocytes required material with high stiffness (~7 kPa), astrocytes medium (1–3 kPa) and neurons soft surfaces (<1 kPa; Thompson & Chan, 2016). These findings suggest that surfaces of certain stiffness can improve the specificity and homogeneity of differentiated neural stem cells. This interpretation is supported by recent findings showing that human induced pluripotent stem cell-derived neural crest stem cells implanted into the rat carotid artery preferentially differentiate to smooth muscle cells in a stiff environment, and to glial cells under softer conditions (Zhu et al., 2019).

Here we investigate how the stiffness of the material and the covering substrate affect bNCSC adhesion, maintenance, and differentiation and show that polyacrylamide (PAA) scaffolds of different stiffness covered with collagen or laminin influence these properties of bNCSCs in 2D cultures.

2 | MATERIALS AND METHODS

2.1 | Culture of bNCSCs

bNCSCs were generated from transgenic mice harboring red fluorescent protein (RFP) under the universal actin promoter (Vintersten et al., 2004) according to previously published protocols (Aldskogius et al., 2009; Hjerling-Leffler et al., 2005). Briefly, the dorsal root ganglia along with boundary caps were mechanically separated from the isolated spinal cord and mechanoenzymatically dissociated using Collagenase/Dispase (1 mg/ml) and DNase (0.5 mg/ml) for 30 min at room temperature. Cells were plated at $0.5–1 \times 10^5$ cells/cm^2 in an N2 medium containing B27 (Gibco) as well as EGF and bFGF (R&D Systems, 20 ng/ml, respectively). After 12 hr, nonadherent cells were removed together with half of the medium before adding fresh medium. The medium was changed every other day (50% of the medium replaced with fresh medium) until neurospheres could be observed after approximately 2 weeks of culture. The cells were frozen and after thawing the dissociated cells were plated in 24 well-dishes or 75 cm^2 flasks for the following experiments.

2.2 | Preparation of polyacrylamide substrates with varying stiffness

Three different types of polyacrylamide (PAA) substrates (0.5, 1, and ~7 kPa) were prepared as described previously (Wang & Pelham Jr., 1998) for bNCSC cultures. Briefly, glass coverslips were treated with 3-aminopropyltrimethoxysilane and 0.5% glutaraldehyde. A solution of 8% acrylamide and varying concentrations of bis-acrylamide (0.1, 0.5, and 0.7%) were mixed. Polymerization was initiated with N,N,N′,N′-tetramethylethylenediamine (TEMED) and ammonium persulfate. Thereafter, 0.2 mg/ml N-sulfosuccinyimidyl-6-(4′-azido-2′-nitrophenylamino) hexanoate (sulfo-SANPAH) dissolved in 10 mM HEPES (pH 8.5) were applied to cover the PAA gel and exposed to 365 nm ultraviolet light for 70 min for photoactivation in 24-well plates. This procedure allows precisely tuned stiffness of the substrate (Tse & Engler, 2010). The PAA was washed three times with phosphate-buffered saline (PBS) to remove excess reagent and incubated with laminin solution (Merck, 1 μg/cm^2), or collagen (Gibco, 200 μg/ml) in each well overnight at 4°C. Before cells were plated, the PAA substrates were soaked in PBS and then in Dulbecco's Modified Eagle Medium (DMEM) at 4°C.

2.3 | Cell adhesion assay

For cell adhesion analysis 1.0×10^4 cells/cm^2 were seeded in each well of a 24-well plate with laminin- or collagen-PAA substrates (1 μg/cm^2), and allowed to attach and grow for 48 hr. Cells were examined and counted in a fluorescent inverted phase-contrast microscope. Thereafter, the cultures were washed three times with PBS to remove nonadherent cells, and the remaining (attached) cells counted.

2.4 | Cell counts

To assess the ability of the different substrates to maintain the bNCSC population, the number of bNCSC cultured on PAA with different stiffness on collagen or laminin surfaces, was counted daily from Day 1 to Day 5. Cultures were photographed and the number of cells estimated by virtual measurement, using ImageJ.

2.5 | Cell differentiation

To estimate bNCSCs differentiation we counted the proportion of cells with an elongated profile (sign of dearly differentiation) in relation to the number of cells with a round profile (sign of undifferentiated state). The proportion of elongated and round cells was determined on Days 1, 2, and 3 on collagen- and laminin-PAA with different stiffness. After 5 days in culture, cells were fixed in 4% paraformaldehyde in PBS for 15 min, and processed for immunocytochemistry. Cells on glass coverslips were pre-incubated with blocking solution (1% bovine serum albumin, 0.3% Triton X-100, and 0.1% sodium azide [NaN3] in PBS) for 45 min at room temperature and then incubated overnight at 4°C with antibodies to glial fibrillary acidic protein (GFAP; mouse,1:400, Merck). Secondary antibodies were Alexa Fluor 488 goat anti-mouse IgG (H + L; 1:200, Life Technologies). Cells were washed twice with PBS, once in distilled water and embedded in 8 μl of mounting medium (50% glycerol in PBS and 100 mM propyl-gallate [Sigma]) on a glass slide.

2.6 | Statistical analysis

Results are presented as means ± *SD*. The difference among samples was considered statistically significant by the confidence interval of 95% ($p < .05$).

3 | RESULTS

3.1 | Cell adhesion

To examine cell adhesion on collagen or laminin surfaces with different PAA stiffness, bNCSCs were cultured for 2 days before washing, and the total number of attached cells was counted. We found that the majority of cells attached to the same efficiency to laminin surfaces regardless of PAA stiffness (Figure 1). The lowest adhesion efficiency was registered on 0.5 kPa PAA covered with collagen. Collagen surface was also less efficient than laminin with a PAA stiffness of 1 kPa. These data show that surface properties (collagen or laminin) are most important for scaffolds with low stiffness, whereas, for higher stiffness, the type of surface is less important for cell adhesion.

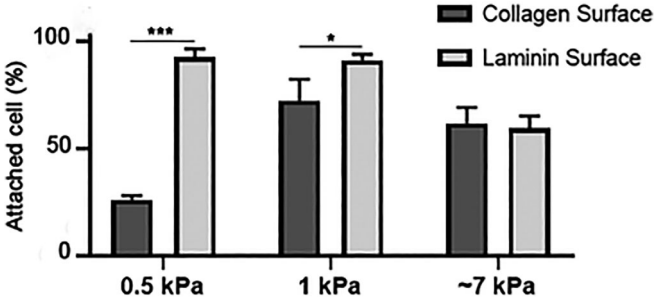

FIGURE 1 bNCSC adhesion rate on PAA scaffold with different stiffness (0.5, 1, and ~7 kPa) covered with collagen or laminin
*$p < .05$; ***$p < .001$

3.2 | Cell number

Cell numbers on collagen or laminin surfaces with different PAA stiffness was assessed every 24 hr from Day 1–5 after attachment. On the collagen surface, a stiffness of ~7 kPa displayed the best ability to maintain bNCSCs (Figure 2a). Between Day 1 and 2, there was a significant increase in the number of cells on this substrate, indicating that the original cell population had grown through proliferation. This increase was abolished after washing the cultures on Day 2. The loss of cells after washing is presumably the result of poor adhesion by a proportion of living cells, which, in turn, could reflect their specific receptor pattern expression. A second increase in the bNCSC population on collagen with a stiffness of ~7 kPa occurred between Day 3 and 4. This rise was, however, not maintained on Day 5, when the bNCSC population was similar in size to Day 1. Collagen surfaces with lower stiffness were less favorable in terms of maintaining the bNCSC population (Figure 2a). On laminin surface with stiffness 0.5 kPa, the cell number was low 24 hr after plating, indicating an early loss of a large proportion of seeded cells (Figure 2b). From that stage, the cell number was maintained, however. For laminin substrate with a stiffness of 1 kPa, the cell number was essentially unchanged throughout the experiment. When the stiffness of laminin was increased to ~7 kPa it was able not only to maintain but also to support growth of the bNCSC population (Figure 2b). A significant increase occurred on this substrate from Day 1 to Day 2, and the loss of cells due to washing on Day 2 was followed by a second significant increase in bNCSC numbers.

3.3 | Cell differentiation

We used transformation of bNCSCs from a round to an elongated shape as evidence of cell differentiation from Day 1 to Day 3. The proportion of elongated cells on collagen-covered ~7 kPa PAA was markedly higher than on collagen surfaces with lower PAA stiffness (Figure 3a). All three types of laminin-covered PAA support the

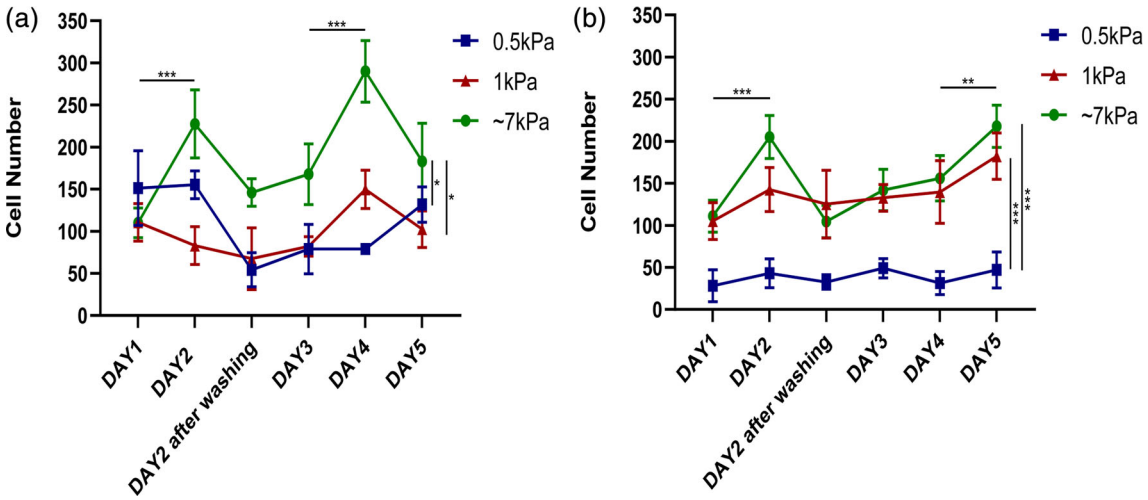

FIGURE 2 Cell number on scaffolds of different PAA stiffness with (a) collagen surface and (b) laminin surface from Day 1–5. $n = 4$;
*$p < .05$; ***$p < .001$

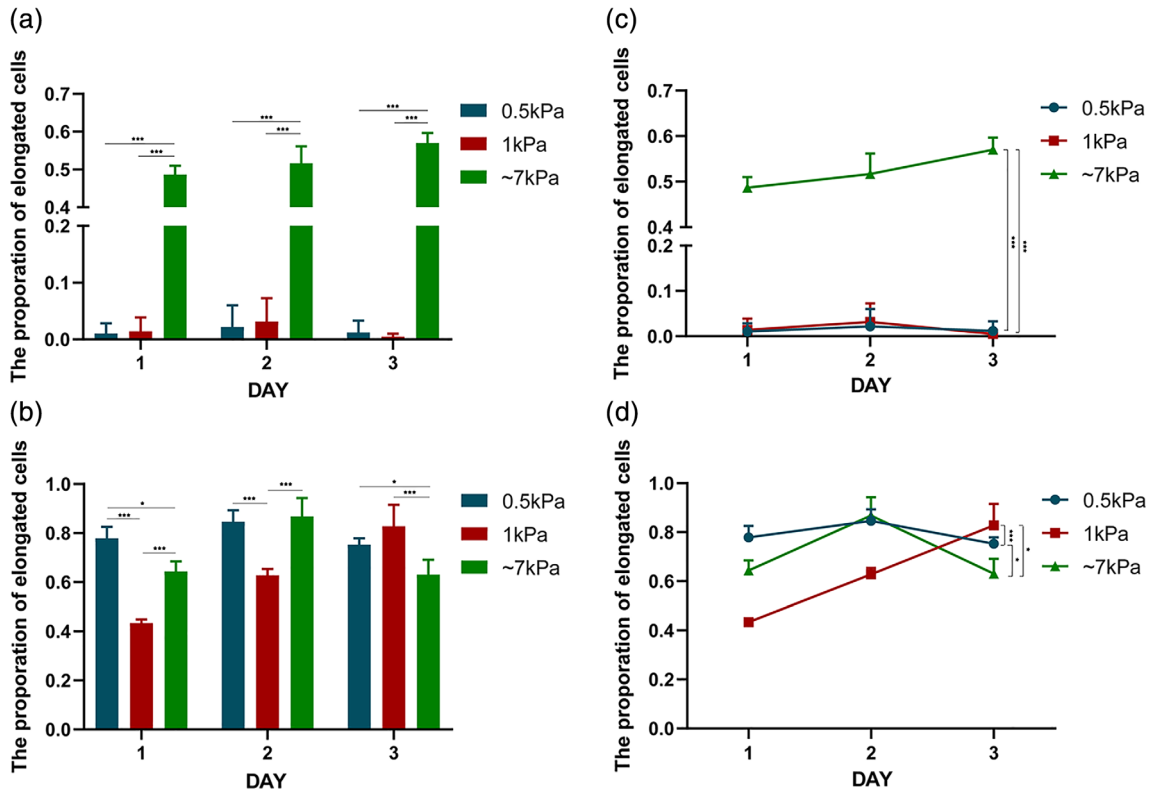

FIGURE 3 Differentiation of bNCSCs on (a,c) collagen and (b,d) laminin surfaces with different scaffold stiffness (0.5, 1, and ~7 kPa). The graphs display the proportion of elongated cells in relation to the total cell number at different time points

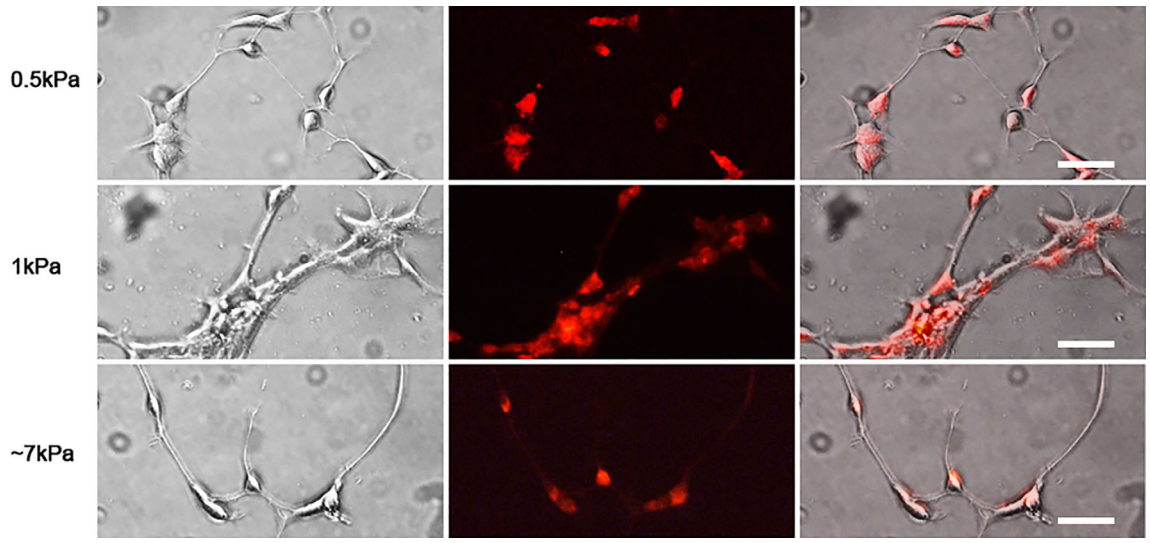

FIGURE 4 Images of elongated bNCSCs, expressing RFP on Day 3 in the scaffold with different stiffnesses. Bar = 25 μm

emergence of a large proportion of elongated cells, although with a somewhat different temporal pattern (Figure 4). Thus, on a PAA stiffness of 0.5 and ~7 kPa PAA the proportion of elongated cells reached a peak on Day 2, and then declined. However, the number of differentiated cells on laminin-covered 1 kPa PAA was larger than on the other two types of stiffness on Day 3. Labeling for the astroglia marker GFAP showed that on laminin a large majority of cells displayed an astroglial phenotype with the largest proportion on 1 kPa PAA stiffness (Figure 5). A few cells were also observed to be GFAP positive, in line with the expression of GFAP in early differentiating astrocytes (Bramanti, Tomassoni, Avitabile, Amenta, & Avola, 2010).

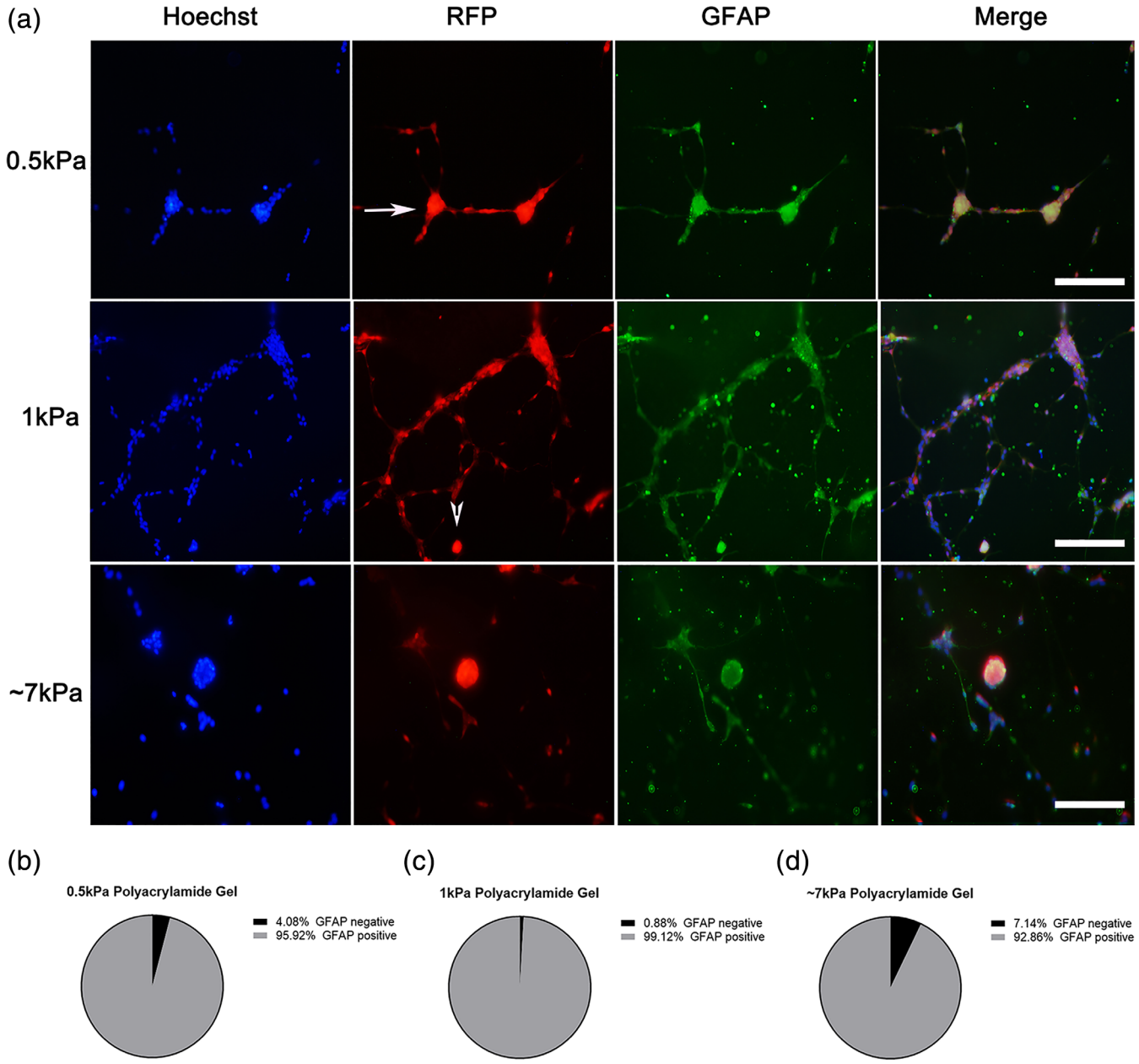

FIGURE 5 Cell differentiation on laminin covered PAA of different stiffness (0.5, 1, and ~7 kPa) for 5 days. (a) GFAP (green) expressing bNCSCs (red) growing on laminin-covered scaffolds. Elongated (arrow) and round (arrowhead) cells are present on all three stiffness variants. Blue—Hoechst nuclear stain. (b–d) Percentage of GFAP-positive cells on laminin-covered 0.5 kPa PAA (b), 1 kPa PAA (c), and ~7 kPa PAA (d). Bar = 25 μm

4 | DISCUSSION

Cells communicate with the environment and other cells through receptors and ion channels, which are located on the surface membrane. Therefore, their ability to attach and elongate on the surface is a crucial factor for their proliferation and differentiation capacity. This is particularly important in view of the broad cell supportive potential of bNCSC in 3D bio-printing for cell survival and support. Furthermore, the interaction between cells and scaffold properties may also display a dynamic temporal pattern that needs to be considered in the design of optimal bioscaffold function.

Here, we investigated at different time points in vitro adhesion, maintenance, and differentiation capacity of bNCSCs on scaffolds with different stiffness covered with collagen or laminin. We show that the scaffold porosity, and its interaction with surface properties of the biomatrix (collagen or laminin), has a major impact on bNCSC attachment, maintenance, and differentiation.

Previous studies have shown that embryonic stem cells grown on stiff substrates maintain their proliferative capacity, but undergo

differentiation when the strength of mechanical signals are reduced (Panciera, Azzolin, Cordenonsi, & Piccolo, 2017). However, there is not a straightforward relationship between mechanical conditions in the environment and stem cell differentiation, since, for example, mesenchymal stem cells (McBeath, Pirone, Nelson, Bhadriraju, & Chen, 2004; Panciera et al., 2017) and neural crest stem cells (Zhu et al., 2019) differentiate along different lineages depending on surrounding mechanical factors. Our findings show that the conditions that favor stem cell differentiation are also markedly affected by the molecular properties of the substrate itself. Thus, whereas soft collagen substrate is unfavorable for bNCSC adhesion, laminin substrates are compatible with a high degree of bNCSC adhesion regardless of scaffold stiffness. At the same time, we have detected that washing on Day 2 essentially reduced the cell number on soft collagen substrates and on stiff laminin substrate. This shows that the ability of bNCSCs to adhere to substrates with markedly different level of stiffness depends on the biomolecules of the substrate—collagen or laminin. Soft collagen substrate also shows inferior capacity to maintain the bNCSC population, indicating a mechanistic relationship between adhesion and bNCSC survival. Furthermore, collagen with 1 kPa PAA, a substrate that provided excellent conditions for bNCSC adhesion, failed to support their elongation, and differentiation.

Laminin provided good conditions for cell adhesion regardless of PAA stiffness, although with a moderate, but still significant, decline with the highest scaffold stiffness. Interestingly, this decline did not negatively affect bNCSC maintenance, which was, in fact, best on high stiffness laminin substrate. All laminin covered stiffness variants also supported differentiation of bNCSC, as evidenced by a large proportion of elongated cells during the culture period, and the presence of more than 90% GFAP expressing cells under all stiffness conditions at the termination of the experiment.

5 | CONCLUSION

Taken together, our finding demonstrates the complex interactions between chemical and physical cues for the outcome of neural stem cell properties and indicates that designing optimal substrate and bioscaffold interactions are fundamental for successful tissue engineering.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Aggarwal, T., Hoeber, J., Ivert, P., Vasylovska, S., & Kozlova, E. N. (2017). Boundary cap neural crest stem cells promote survival of mutant SOD1 motor neurons. *Neurotherapeutics*, *14*(3), 773–783. https://doi.org/10.1007/s13311-016-0505-8

Aldskogius, H., Berens, C., Kanaykina, N., Liakhovitskaia, A., Medvinsky, A., SandelinM, S. S., … Kozlova, E. N. (2009). Regulation of boundary cap neural crest stem cell differentiation after transplantation. *Stem Cells*, *27*(7), 1592–1603. https://doi.org/10.1002/stem.77

Aquino, J. B., Hjerling-Leffler, J., Koltzenburg, M., Edlund, T., Villar, M. J., & Ernfors, P. (2006). In vitro and in vivo differentiation of boundary cap neural crest stem cells into mature Schwann cells. *Experimental Neurology*, *198*(2), 438–449 Epub 2006 Jan 25.

Bramanti, V., Tomassoni, D., Avitabile, M., Amenta, F., & Avola, R. (2010). Biomarkers of glial cell proliferation and differentiation in culture [Review]. *Frontiers in Bioscience (Scholar Edition)*, *2*, 558–570.

Burdick, J. A., & Vunjak-Novakovic, G. (2009). Engineered microenvironments for controlled stem cell differentiation [Review]. *Tissue Engineering. Part A*, *15*(2), 205–219. https://doi.org/10.1089/ten.tea.2008.0131

Grapensparr, L., Vasylovska, S., Li, Z., Olerud, J., Jansson, L., Kozlova, E., & Carlsson, P. O. (2015). Co-transplantation of human pancreatic islets with post-migratory neural crest stem cells increases β-cell proliferation and vascular and neural regrowth. *The Journal of Clinical Endocrinology and Metabolism*, *100*(4), E583–E590. https://doi.org/10.1210/jc.2014-4070

Grouwels, G., Vasylovska, S., Olerud, J., Leuckx, G., Ngamjariyawat, A., Yuchi, Y., … Heimberg, H. (2012). Differentiating neural crest stem cells induce proliferation of cultured rodent islet beta cells. *Diabetologia*, *55*(7), 2016–2025. https://doi.org/10.1007/s00125-012-2542-0

Hjerling-Leffler, J., Marmigère, F., Heglind, M., Cederberg, A., Koltzenburg, M., Enerbäck, S., & Ernfors, P. (2005). The boundary cap: A source of neural crest stem cells that generate multiple sensory neuron subtypes. *Development*, *132*(11), 2623–2632.

Lau, J., Vasylovska, S., Kozlova, E. N., & Carlsson, P. O. (2015). Surface coating of pancreatic islets with neural crest stem cells improves engraftment and function after intraportal transplantation. *Cell Transplantation*, *24*(11), 2263–2272. https://doi.org/10.3727/096368915X686184

McBeath, R., Pirone, D. M., Nelson, C. M., Bhadriraju, K., & Chen, C. S. (2004). Cell shape, cytoskeletal tension, and RhoA regulate stem cell lineage commitment. *Developmental Cell*, *6*(4), 483–495.

Ngamjariyawat, A., Turpaev, K., Vasylovska, S., Kozlova, E. N., & Welsh, N. (2013). Co-culture of neural crest stem cells (NCSC) and insulin producing beta-TC6 cells results in cadherin junctions and protection against cytokine-induced beta-cell death. *PLoS One*, *8*(4), e61828. https://doi.org/10.1371/journal.pone.0061828

Ngamjariyawat, A., Turpaev, K., Welsh, N., & Kozlova, E. N. (2012). Coculture of insulin-producing RIN5AH cells with neural crest stem cells protects partially against cytokine-induced cell death. *Pancreas*, *41*(3), 490–492. https://doi.org/10.1097/MPA.0b013e31823fcf2a

Olerud, J., Kanaykina, N., Vasylovska, S., King, D., Sandberg, M., Jansson, L., & Kozlova, E. N. (2009). Neural crest stem cells increase beta cell proliferation and improve islet function in co-transplanted murine pancreatic islets. Erratum in: *Diabetologia*. 2010;53(2):396. Vasilovska, S [corrected to Vasylovska, S]. *Diabetologia*, *52*(12), 2594–2601. https://doi.org/10.1007/s00125-009-1544-z

Panciera, T., Azzolin, L., Cordenonsi, M., & Piccolo, S. (2017). Mechanobiology of YAP and TAZ in physiology and disease. *Nature Reviews. Molecular Cell Biology*, *18*(12), 758–770. https://doi.org/10.1038/nrm.2017.87

Radomska, K. J., & Topilko, P. (2017). Boundary cap cells in development and disease [Review]. *Current Opinion in Neurobiology*, *47*, 209–215. https://doi.org/10.1016/j.conb.2017.11.003

Schizas, N., König, N., Andersson, B., Vasylovska, S., Hoeber, J., Kozlova, E. N., & Hailer, N. P. (2018). Neural crest stem cells protect spinal cord neurons from excitotoxic damage and inhibit glial activation by secretion of brain-derived neurotrophic factor. *Cell and Tissue Research*, *372*(3), 493–505. https://doi.org/10.1007/s00441-018-2808-z

Thompson, R., & Chan, C. (2016). Signal transduction of the physical environment in the neural differentiation of stem cells. *Technology (Singapore World Science)*, *4*(1), 1–8. https://doi.org/10.1142/S2339547816400070

Trolle, C., König, N., Abrahamsson, N., Vasylovska, S., & Kozlova, E. N. (2014). Boundary cap neural crest stem cells homotopically implanted to the injured dorsal root transitional zone give rise to different types of neurons and glia in adult rodents. *BMC Neuroscience*, *15*, 60. https://doi.org/10.1186/1471-2202-15-60

Tse, J. R., & Engler, A. J. (2010). Preparation of hydrogel substrates with tunable mechanical properties. *Current Protocols in Cell Biology*, *Chapter 10*, Unit 10.16.

Vintersten, K., Monetti, C., Gertsenstein, M., Zhang, P., Laszlo, L., Biechele, S., & Nagy, A. (2004). Mouse in red: Red fluorescent protein expression in mouse ES cells, embryos, and adult animals. *Genesis*, *40*(4), 241–246.

Wang, X., Xie, B., Qi, Y., Wallerman, O., Vasylovska, S., Andersson, L., … Welsh, N. (2016). Knock-down of ZBED6 in insulin-producing cells promotes N-cadherin junctions between beta-cells and neural crest stem cells in vitro. *Scientific Reports*, *6*, 19006. https://doi.org/10.1038/srep19006

Wang, Y. L., & Pelham, R. J., Jr. (1998). Preparation of a flexible, porous polyacrylamide substrate for mechanical studies of cultured cells. *Methods in Enzymology*, *298*, 489–496.

Zhu, Y., Li, X., Janairo, R. R. R., Kwong, G., Tsou, A. D., Chu, J. S., … Li, S. (2019). Matrix stiffness modulates the differentiation of neural crest stem cells in vivo. *Journal of Cellular Physiology*, *234*(5), 7569–7578. https://doi.org/10.1002/jcp.27518

Zujovic, V., Thibaud, J., Bachelin, C., Vidal, M., Coulpier, F., Charnay, P., … Baron-Van Evercooren, A. (2010). Boundary cap cells are highly competitive for CNS remyelination: Fast migration and efficient differentiation in PNS and CNS myelin-forming cells. *Stem Cells*, *28*(3), 470–479. https://doi.org/10.1002/stem.290

Zujovic, V., Thibaud, J., Bachelin, C., Vidal, M., Deboux, C., Coulpier, F., … Baron-Van Evercooren, A. (2011). Boundary cap cells are peripheral nervous system stem cells that can be redirected into central nervous system lineages. *Proceedings of the National Academy of Sciences of the United States of America*, *108*(26), 10714–10719. https://doi.org/10.1073/pnas.1018687108

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