DYNAMICS OF MARKERS OF STRUCTURAL AND FUNCTIONAL STATE OF THE ENDOTHELIUM IN SUBCUTANEOUS IMPLANTATION OF SCAFFOLDS OF POLYCAPROLACTONE AND VATERITE

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Aims

The aim of this work was to evaluate the dynamics of markers of the structural and functional state of the endothelium in subcutaneous implantation of scaffolds of polycaprolactone and vaterite as compared to non-biocompatible matrices.

Materials and Methods

In the experiment in albino rats, the changes in serum concentrations of C-reactive protein, vascular endothelial growth factor, syndecan 1 and VE-cadherin were studied in the subcutaneous implantation of scaffolds of polycaprolactone and vaterite compared to non-biocompatible matrices.

Results

It has been established that the absence of biocompatibility of scaffolds is manifested as a systemic inflammatory response and prolonged proangiogenic activity accompanied by the formation of an inflammatory phenotype of endotheliocytes. In the implantation of scaffolds of polycaprolactone, the pattern of systemic inflammatory response is consistent with that of sham operated animals, indicating biocompatibility of these matrices. The increase in vascular endothelial growth factor concentration soon after implantation, in the absence of evidence of endothelial glycocalyx damage, characterises the high angiogenic potential of the scaffolds of polycaprolactone and vaterite.

Conclusions

The most informative element for predicting vascularisation of scaffolds in subcutaneous implantation tests is the ratio of the change in serum concentrations of vascular endothelial growth factor and syndecan 1, which enables the evaluation of the angiogenic potential of the matrix from the seventh day after subcutaneous implantation until the morphological evidence of new vessel formation.

Keywords: scaffolds, vascularisation, endothelium, inflammation, regeneration.

INTRODUCTION

Today's regenerative medicine sees prospects in 3D scaffolds that function as intercellular matrices to support the migration, proliferation, and directed differentiation of cells, thus accelerating the reparation processes [1]. Regeneration requires adequate tissue nourishment, which in the case of 3D matrices is enabled by vessels growing into the scaffold [2]. With respect to scaffolds used to stimulate the regeneration of bone tissue, what makes intra-matrix vessels so important is the multifaceted role vessels play in the migration of osteoclasts and osteoblasts, as well as in the mineralization of the newly formed bone tissue and its subsequent remodeling. If new vessels fail to emerge in the scaffold, it will hinder osteogenesis, i.e. reduce the osteoinductive properties of the implanted matrix [3]. Therefore, the angiogenic characteristics of the scaffold contribute substantially to its osteoinductive properties and the general regenerative potential alike. This is why the current situation calls for research into the vascularization of mineral-based matrices and the factors regulating the process, as such research will help predict and modulate the regeneration-boosting properties of scaffolds.

Regardless of the application, scaffolds must be biocompatible [4]. The intensity, duration, and specifics of the inflammatory response to implanting are the key indicators of biocompatibility. Notably, some inflammation mediators induce angiogenesis, thus being crucial to the effective scaffold vascularization. At the same time, intense inflammation inhibits scaffold vascularization and cellular population [5].

Angiogenic reactions are based on the migration and proliferation of endothelial cells. For this study, research into how scaffold biocompatibility and structural/functional endothelial changes correlate is of theoretical and practical interest, in particular with respect to the predictive value of the markers of angiogenic reactions in endothelial cells, which is what defines the focus of this research.

The research goal is to evaluate the dynamics of the structural and functional endothelial markers as affected by subcutaneous implantation of polycaprolactone and vaterite scaffolds in comparison to non-biocompatible matrices.

MATERIAL AND METHODS

The research design involved 54 animals split at random into four groups: control (8 rats), comparison (12 sham-operated rats), negative control (17 rats with the implanted non-biocompatible scaffolds: polycaprolactone matrices containing native ovalbumin), and experimental (17 animals with the implanted polycaprolactone and vaterite scaffolds). Pursuant to the guidelines of Razumovsky Saratov State Medical University's Ethics Committee (Minutes No. 6 of February 6, 2018), invasive manipulations on rats were performed with general anesthesia carried out by combined intramuscular administration of xylazine (Interchemie, the Netherlands), and telazol (Zoetis, Spain). Rats were sacrificed by overdosing these medications.

The implanted matrices were made by the Education and Research Institute of Nanostructures and Biosystems. Subcutaneous implantation tests lasting 7 or 21 days were run following the method described in [4, 6]. On Days 7 or 21, the cardiac puncture was performed to sample 5 ml of blood to produce serum.

ELISA tests were used to detect the following in the experimental animals' serum: C-reactive protein (CRP) with the Rat C-Reactive Protein ELISA kits (eBioscience, USA) to evaluate the systemic inflammatory response; vascular endothelial growth factor (VEGF) with the Rat VEGF Immunoassay (R&D Systems, USA) to evaluate angiogenesis-regulating mechanisms; soluble forms of syndecan-1 and VE-cadherin with the ELISA Kit for Syndecan 1 Rat and ELISA Kit for Cadherin 5 Rat (CloudClone Corp, USA) to evaluate damage sustained by endotheliocyte glycocalyx and the stability of the microvascular bed. ELISA tests were run on an Anthos 2020 microplate reader (Biochrom, UK) exactly as instructed by the kit manufacturers.

The collected data were processed statistically in Statistica 10.0 software and presented as median and interquartile ranges because the data were not normally distributed. Mann-Whitney tests were run for pairwise comparison to calculate the confidence level (p=0.05 as the threshold).

RESULTS

The research found out that on Day 7 of subcutaneous matrix implantation, the comparison rats had their CRP level increased by 18.7%. CRP in sham-operated animals did not differ significantly from that in intact animals by Day 21, see the Table.

On Day 7, the comparison rats had a 1.7 times higher VEGF concentration than the controls, a sign of proangiogenic activity induced by tissue injury. By Day 21 after the surgeries, VEGF levels in the comparison animals normalized and did not differ significantly from that in the controls, a sign of active angiogenesis being over. On Days 7 and 21 after the sham surgeries, comparison rats did not have significantly different concentrations of soluble endothelial cell glycocalyx components compared to the controls, see the Table. Therefore, implantation-free surgery induced a transient mild increase in CRP and VEGF concentrations in blood as recorded on Day 7. Normalization of the biochemical parameters by Day 21 indicated the completion of angiogenic processes and the stabilization of the vascular bed; it also corresponded to the lack of systemic inflammatory response signs. This is in line with the earlier findings of the dynamic microcirculatory-bed blood flow monitoring and with the morphological characteristics of surgeries in the scope of subcutaneous implantation testing [6].

In negative controls, CRP concentration rose on Day 7 after implanting non-biocompatible ovalbumin-impregnated scaffolds; the difference was significant against the controls and the sham-operated rats alike. On Day 21, CRP concentration dropped in negative controls compared to Day 7 values; however, it was still 1.25 times and 1.17 times higher than that of intact and sham-operated animals, respectively, see the Table.

Table 1

Concentration of the structural and functional endothelial markers in the experimental animals' serum

Groups	CRP, mg/l	VEGF, pg/ml	Syndecan-1, ng/ml	VE-cadherin, pg/ml
Controls (n=8)	101 (96; 114)	9.4 (7.3; 15.7)	1.11 (0.82; 1.51)	56.18 (54; 60.54)
Comparison group, Day 7 (n=6)	120 (118; 126) p1<0.05	16.2 (14.7; 18.8) p1<0.05	1.45 (0.91; 1.88) p1>0.05	56.18 (54; 60.55) p1>0.05
Comparison group, Day 21 (n=6)	108 (106; 117) p1>0.05 p2>0.05	8.9 (7.3; 12.6) p1>0.05 p2>0.05	1.2 (0.97; 1.84) p1>0.05 p2>0.05	57.27 (54; 60.55) p_1>0.05 p_2>0.05
Negative controls, Day 7 (n=8)	168 (131; 203) p1<0.05 p3<0.05	30.8 (26.1; 34.4) p1<0.05 p3<0.05	2.31 (1.91; 2.67) p1<0.05 p3<0.05	60.55 (54; 62.73) p1>0.05 p3<0.05
Negative control, Day 21 (n=9)	127 (125; 129) p_1<0.05 p_2<0.05 p_3<0.05	54.2 (23; 69.2) p_1<0.05 p_2>0.05 p_3<0.05	2.3 (1.96; 2.4) p_1<0.05 p_2>0.05 p_3<0.05	58.4 (56.2; 62.6) p_1>0.05 p_2>0.05 p_3>0.05
Experimental group, Day 7 (n=8)	126 (108; 129) p1<0.05 p3>0.05 p4<0.05	50 (32.3; 66.2) p1<0.05 p3<0.05 p4<0.05	1.31 (1.11; 1.78) p1>0.05 p3>0.05 p4<0.05	57.2 (52.9; 60.55) p1>0.05 p3>0.05 p4>0.05
Experimental group, Day 21 (n=9)	106 (102; 109) p_1>0.05 p_2>0.05 p_3>0.05 p_4<0.05	11.5 (9.5; 16.7) p_1>0.05 p_2<0.05 p_3>0.05 p_4<0.05	1.37 (0.63; 2.04) p_1>0.05 p_2>0.05 p_3>0.05 p_4<0.05	57.3 (53; 62.7) p_1>0.05 p_2>0.05 p_3>0.05 p_4>0.05

Note: The results are shown as the median and the interquartile range. $p_{1,2,3,4}$ are statistical significance values of the difference against the controls, Day 7 data, the comparison group, and the negative control on the same day, respectively.

A systemic inflammatory response featuring a higher CRP concentration in blood might be due to local leukocyte infiltration of the ovalbumin-impregnated matrix itself and its perifocal zone as discovered by earlier morphological studies [7]; this infiltration is associated with a hyperproduction of proinflammatory cytokines and indicates the non-biocompatibility of the scaffold. Negative controls had 1.9 times higher VEGF concentration than that of the comparison rats on Day 7, see the Table. By Day 21, non-biocompatible matrices tended to further increase in VEGF concentration, a sign of prolonged and pronounced activation of angiogenesis. Literature data suggest that prolonged hyperproduction of VEGF does not lead to scaffold vascularization; it is likely associated with the vascularization of the growing connective tissue that creates a separating barrier around the matrix [5]. Therefore, increase blood levels of VEGF persisting up to 21 days after subcutaneous implantation testing are not a favorable condition for scaffold vascularization.

No significant change in the concentration of VE-cadherin was identified in the negative controls. On Day 7 and Day 21 alike, these animals had the concentration of syndecan-1 two times higher compared to the controls (see the Table), a sign of glycocalyx degradation in the endothelial cells. The intensifying shedding of soluble syndecan-1 signifies the forming of the inflammatory endothelial cell phenotype and indicates that the adhesive properties and barrier functions are jeopardized [8-10]. Given that an increase in syndecan-1 was observed as early as on Day 7 after implanting non-biocompatible matrixes, measuring this marker in the blood can help predict scaffold non-vascularization.

On Day 7 after implanting polycaprolactone and vaterite matrices, the experimental white rats and the comparison rats alike had higher CRP concentrations than the controls. CRP levels normalized in the experimental group by Day 21. The CRP concentration change in the experimental group was no more significant than that in

the comparison group, meaning that such change was injury-induced. On Day 7 of implanting polycaprolactone and vaterite scaffolds, experimental animals had the VEGF concentration 3.1 times higher of the comparison rats, 1.6 times higher than that of the negative controls. By Day 21, VEGF concentration dropped in the experimental rats and did not differ significantly anymore from that of the controls, see the Table. Morphological methods earlier found that by Day 21, polycaprolactone and vaterite matrices were effectively vascularized [7]. Thus, a higher early post-implantation VEGF concentration gives a favorable scaffold vascularization prognosis.

Unlike the negative controls, the experimental rats did not have a significantly higher syndecan-1 concentration (see the Table), which showed the endotheliocyte glycocalyx did not sustain damage. The findings suggest that it is not a change in the VEGF concentration in blood, but the ratio of change in the VEGF and syndecan-1 concentrations in serum that is most valuable for predicting the angiogenic potential of scaffolds in subcutaneous implantation tests. Such predictions can be made biochemically as early as on Day 7 whereas literature data suggests [4] that the morphological evaluation of scaffold vascularization is only possible as early as on Day 14.

CONCLUSIONS

1. The complete normalization of CRP, VEGF, and soluble glycocalyx concentrations in sham-operated animals completely excludes surgery-associated tissue injury as a factor affecting the state of endothelial cells on Day 21 after implanting scaffolds.

2. Implanting non-biocompatible scaffolds triggers a pronounced systemic inflammatory response and prolonged activation of angiogenesis associated with glycocalyx degradation and an inflammatory endotheliocyte phenotype being formed.

3. Implanting polycaprolactone matrices triggers a systemic inflammatory response identical to that in sham-operated animals, meaning such matrices are biocompatible. A higher VEGF concentration early after implanting characterizes the high angiogenic potential of polycaprolactone and vaterite matrices provided there are no signs of damage to endothelial glycocalyx.

4. The angiogenic potential of scaffolds can be predicted from the endothelial cell state markers as early as on Day 7 before morphological signs of vascularization emerge. The ratio of change in the serum concentrations of VEGF and syndecan-1 is the most valuable marker for predicting the angiogenic potential of scaffolds in subcutaneous implantation tests.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest

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