Immunogenicity Assessment of Pro- and Peptide Hormone Release in Engineered Tissues

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Introduction

Engineered tissue, releasing peptide hormones become important substitutes for surgery and implantation. These scaffolds confer mechanical stability, topical release of growth factors which in turn induce local cell growth of “new” tissue. Implants used in tissue engineering comprise:

- A bioresorbable three dimensional scaffold conferring mechanical properties,
- Growth factors improving the healing rate,
- Cells synthesizing tissue matrix.

The implant in this study is a soft scaffold improving tissue repair. A pro-hormone -linked to the scaffold by a peptide bond- is gradually released upon cellular invasion. Once converted to its active form, the hormone enhance tissue repair.

It is crucial to assess the immunogenicity of such engineered tissue, as immune response may alter the treatment efficacy and patient safety:

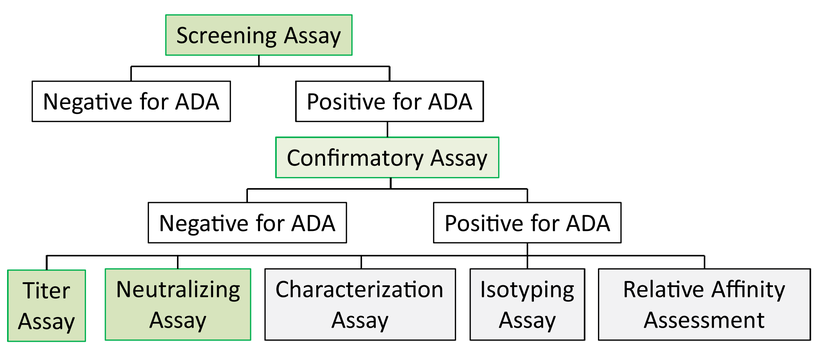
- Anti-drug antibodies (ADA) and neutralizing Ab’s (nAb) specific to the pro- and the released hormone could hamper the re-growth of new tissue and render the scaffold non-functional,
- Cross-reactivity of the ADA to a non-redundant endogenous counterpart of the therapeutic peptide could have deleterious effects.

Here we show the challenges/solutions and special considerations when analyzing the immunogenicity of pro- and peptide hormone release in engineered tissues.

Analytical Methods

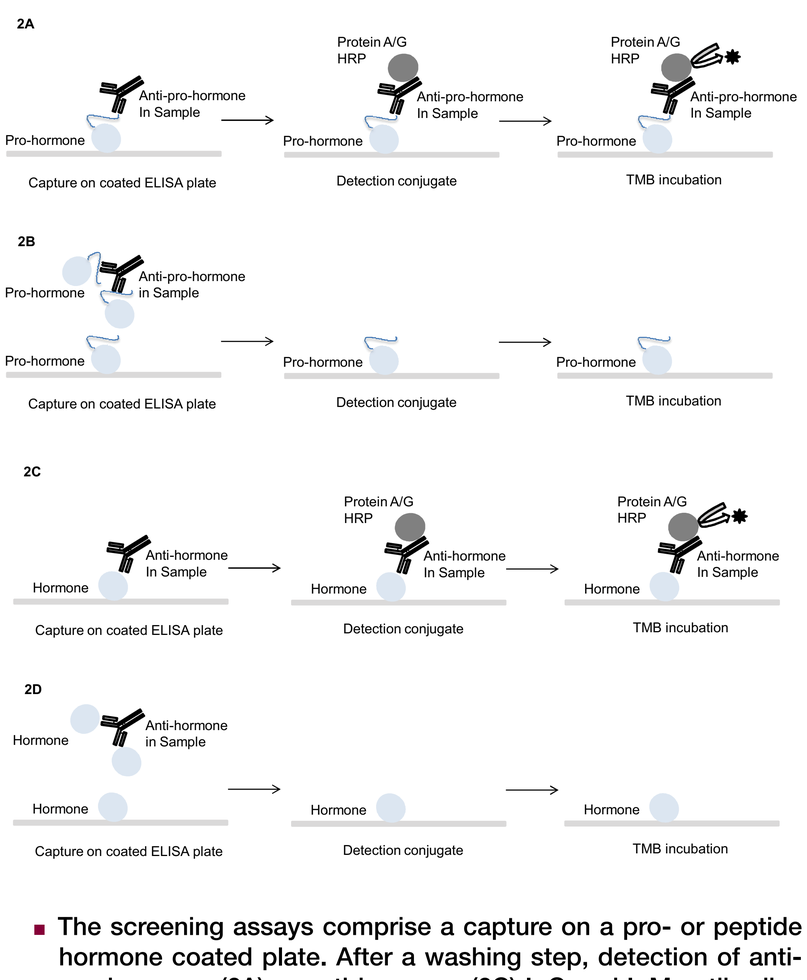
Two immunogenicity assays were developed to detect antibodies against the pro-hormone and the hormone itself. A tiered approach comprising the development of screening, confirmatory and titer formats was applied (Figure 1)

Figure 1: Tiered approach for Immunogenicity assessment



Screening and confirmatory assays for the pro-hormone and the hormone are presented in Figures 2A,-2B and 2C-2D respectively.

Figure 2: Assay Formats



- The screening assays comprise a capture on a pro- or peptide hormone coated plate. After a washing step, detection of anti-pro-hormone (2A) or anti-hormone (2C) IgG and IgM antibodies are detected by addition of protein A/G HRP conjugate.
- In the confirmatory assay, exogenous pro- or peptide hormone added to samples competes with coated pro- or peptide hormone used for capture on the assay plate. Variation of percent inhibitions in a therapeutic-naïve population is taken into consideration for confirming a positive response (2B and 2D), meaning presence of ADA against pro or peptide hormone.

Analytical Challenges and Solutions

1: Coating and Detection Conditions

During the early method development to detect anti-hormone antibodies a poor signal to noise ratio was observed.

Solution: The coating and detection conditions were optimized for three reference items:

- A rabbit anti-Human hormone polyclonal Ab,
- A goat anti-Human hormone polyclonal IgG Ab,
- A purified recombinant rabbit monoclonal IgG anti-Human hormone Ab

The best conditions -coating conditions C and HRP conjugate dilution Y- were selected for further development (Figure 3).

Figure 3: Coating and Detection Optimization

		Signal/blank ratio								
		Coating conditions A			Coating conditions B			Coating conditions C		
	Ref item (ng/mL)	Ref. item 1	Ref. item 2	Ref. item 3	Ref. item 1	Ref. item 2	Ref. item 3	Ref. item 1	Ref. item 2	Ref. item 3
HRP conjugate dilution X	1000	3.0	6.8	20.2	4.8	11.5	22.6	4.3	10.8	19.8
	500	2.2	3.9	11.5	2.7	5.7	11.0	3.3	7.2	12.8
	100	1.0	1.4	2.8	1.3	2.1	3.4	1.5	2.2	3.9
HRP conjugate dilution Y	1000	3.0	6.1	22.0	5.4	11.3	22.7	6.6	15.4	31.5
	500	1.7	3.0	10.1	1.9	3.8	8.1	4.0	8.1	17.4
	100	1.2	1.4	3.5	1.1	1.4	2.9	1.5	2.0	4.0

Ref. item 1 Rabbit anti-Human hormone polyclonal Ab
Ref. item 2 Goat anti-Human hormone polyclonal IgG Ab
Ref. item 3 Purified recombinant rabbit monoclonal IgG Ab against hormone

2: Minimum Required Dilution Assessment

Matrix effect -high signal variation between individuals- was observed during early method development to detect anti-hormone antibodies in naïve samples.

Solution: The minimum required dilution (MRD) was defined by comparing specific signal from spiked (Figure 4A) vs from unspiked samples (Figure 4B). MRD 20 was selected (Figure 5).

Figure 4: Minimum Required Dilution Optimization

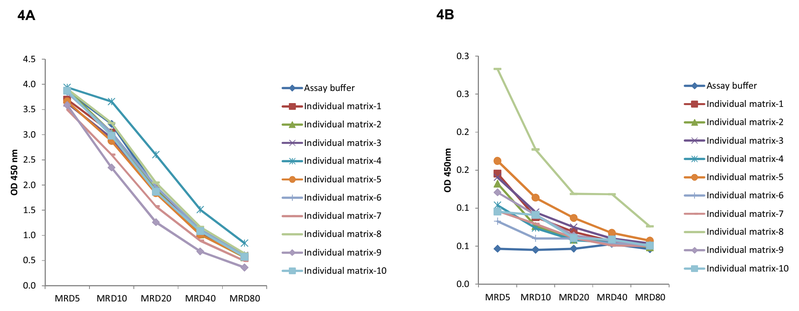


Figure 5: Comparison of Minimum Required Dilutions

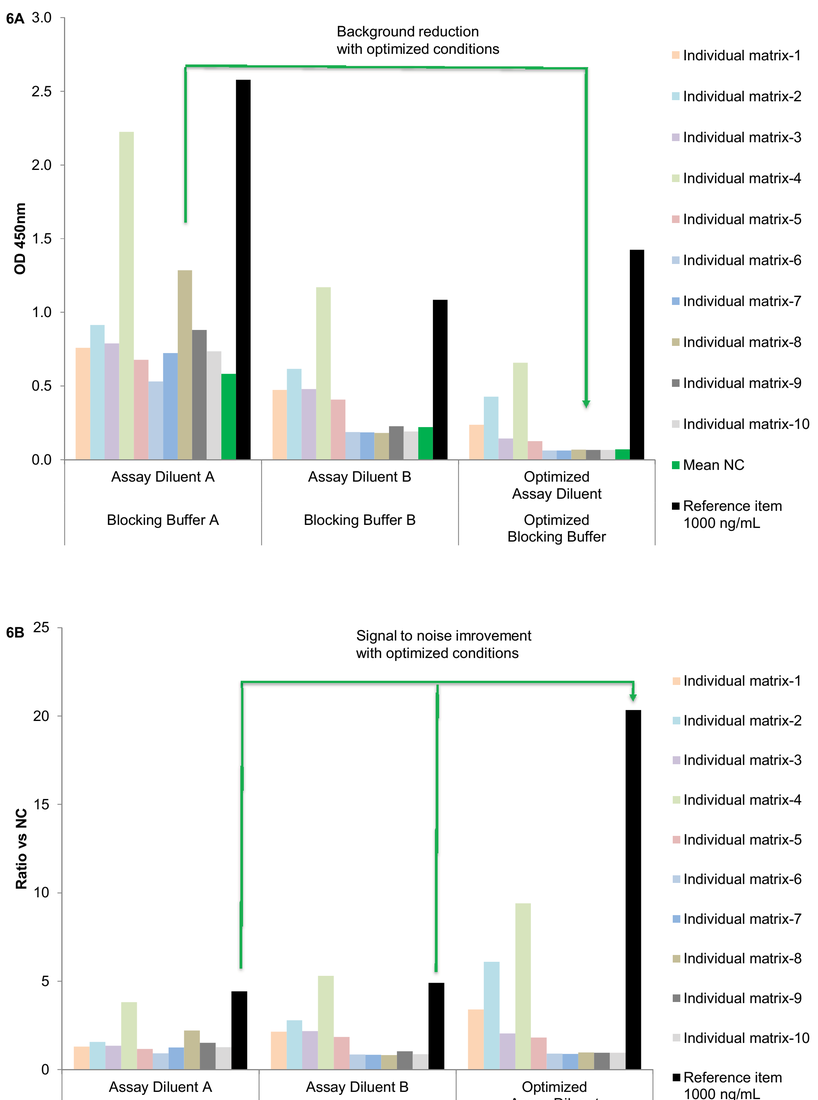
	MRD5	MRD10	MRD20	MRD40	MRD80
Mean spiked (1000ng/mL)	3.761	2.997	1.892	1.061	0.584
Mean unspiked	0.128	0.090	0.069	0.062	0.053
STDEV unspiked	0.061	0.034	0.020	0.019	0.008
Cut point estimation	0.239	0.152	0.105	0.097	0.068
Mean spiked/estimated Cut point	15.706	19.662	18.081	10.917	8.593

3: Background Reduction

A strong background signal (elevated OD without pro-hormone or hormone present) was observed when using blocking and diluent buffers containing animal proteins.

Solution: The background was successfully reduced (Figure 6A) and the signal to noise ratio improved by using protein-free blocking and diluent buffers (Figure 6B).

Figure 6: Blocking and Diluent Buffers Optimization

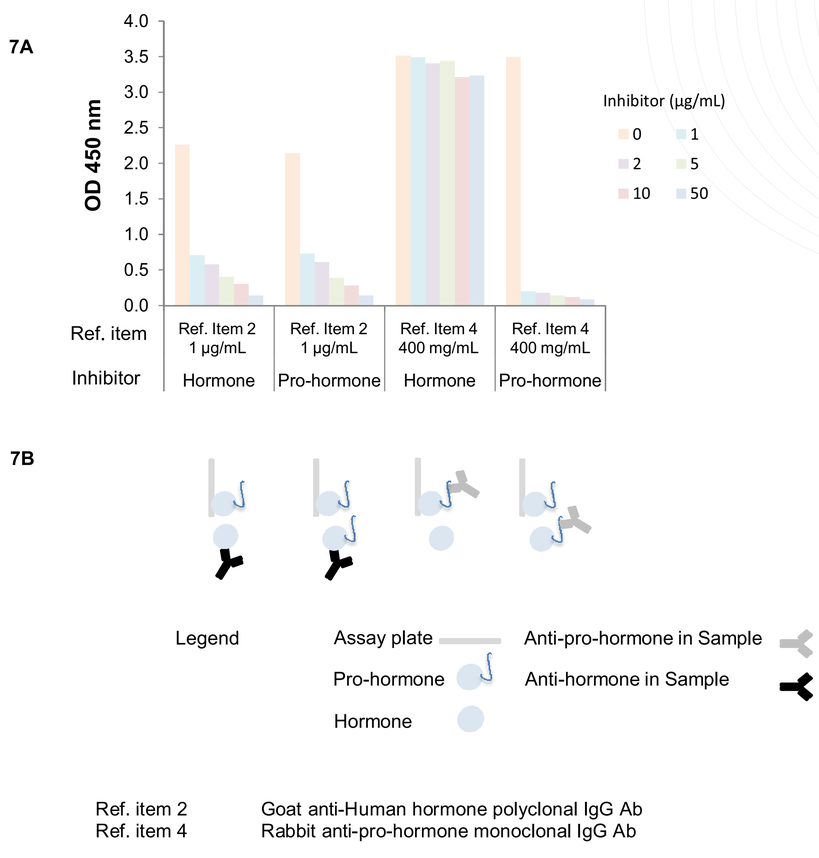


4: Differential Immunogenicity Analysis of ADA’s Raised Against the Pro -Hormone Versus ADA Raised Against the Hormone

With the anti-pro-hormone format, anti-hormone antibodies will be detected together with antibodies against the specific sequence of the pro-hormone.

Antibodies against the specific sequence of the pro-hormone will be assessed indirectly, by data comparison with the anti-hormone assay (Figure 7).

Figure 7: Indirect Immunogenicity Assessment of the Specific Sequence of the Pro-hormone



Ref. item 2 Goat anti-Human hormone polyclonal IgG Ab
Ref. item 4 Rabbit anti-pro-hormone monoclonal IgG Ab

Results

Results summarizing assay sensitivities and cut point estimations are depicted in Figure 8.

Assays were set up using monoclonal and polyclonal antibodies as reference items. The anti-pro-hormone assay was qualified using a monoclonal antibody specific for the pro-peptide, whereas the anti-hormone assay was developed with a polyclonal antibody. Sensitivities below 100 ng/mL were assessed for both ADA assays (5.76 and 49.0 ng/mL for the anti-pro-hormone and anti-hormone assays respectively). Selectivity and recovery was assessed and was within acceptance criteria for both assays. No hook effect was observed for both assays.

Figure 8: Qualification Data

	Anti-pro-hormone assay	Anti-hormone assay
Reference item	Rabbit anti-pro-hormone monoclonal IgG Ab	Goat anti-hormone polyclonal IgG Ab
Sensitivity screening assay (ng/ml)	5.76	49.0
Sensitivity confirmatory assay (ng/ml)	6.94	32.0
Screening Cp (OD at 450 nm)	0.183	0.128
Specificity Cp (% inhibition)	54.5	40.0

Discussion and Conclusions

When biologically active molecules -such as hormones- are used in tissue engineering, antibodies (ADA) raised against these therapeutic products might have deleterious effects on the scaffold functionality and the patient safety. In consequence a thorough analysis of these ADA is mandatory.

In the present study we have successfully developed highly sensitive bioanalytical assays for the detection of ADA species against the pro-hormone and the hormone used in a scaffold for tissue engineering. These assays will enable the optimization of the scaffold for tissue repair.

References

Assay Development and Validation for Immunogenicity Testing of Therapeutic Protein Products. Guidance for Industry. Draft Guidance. US FDA 2016