

## Supplementary Material for:

# Understanding matrix stiffness in vinyl polymer hydrogels: Implications in bone tissue engineering

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- # Equal contribution

## Material and Methodology

### Materials

Alkaline phosphatase kit (Colorimetric) [cat no- ab83369] (ALP assay buffer I, p-nitrophenolphosphate, stop solution)

### ALP activity test

The enzymatic activity of the ALP encapsulated on the hydrogels is evaluated on 0 day and 7 day. 7 days treated scaffolds implies the 30 mins incubation of scaffolds with ALP followed by 7 days treatment with  $\beta$ GP.

After 7 days of treatment the scaffolds were washed in milli-Q 3 times and then freeze-dried for 72 hours. The scaffolds were broken into pieces inside a 1.5 mL vial and re-suspended in 100  $\mu$ L of assay buffer. The vials went for sonication for 4 cycles of 5 minutes each. After complete sonication the vials were centrifuged at 15000 $\times$ g for 15 minutes at 4<sup>0</sup>C. The supernatant were collected and kept in ice. 80  $\mu$ L of supernatant was taken in a well of 96 well plate and 50  $\mu$ L of

pNPP solution was added to it. The samples were left for 60 minutes at room temperature and reaction was carried out in dark condition. After 60 minutes, stop solution was given to the sample to stop the reaction and then the absorbance of the sample was studied at 405 nm.

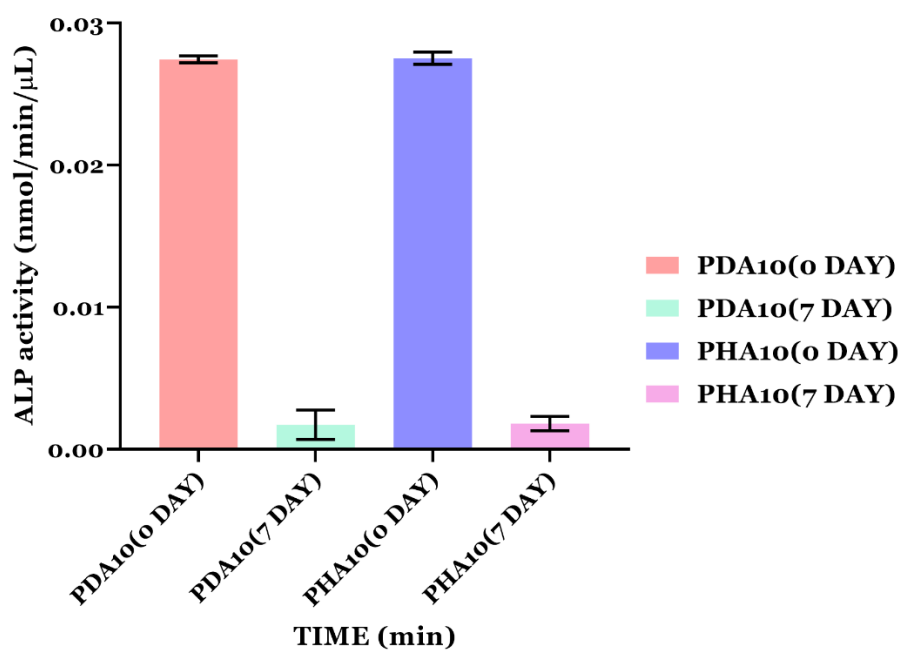
<b>Sample</b>	<b>Amount (<math>\mu</math>l)</b>
<b>pDMAEMA(PD)/</b>	350/525
<b>pHPMA(PH)</b>	
<b>HEMA</b>	200/225
<b>H<sub>2</sub>O</b>	400/300
<b>HA/LAP</b>	0/40/200/400
<b>APS</b>	30/45
<b>TEMED</b>	3.09/4.635

**Table 1-** Composition of the reagents used for hydrogel preparation

<b>Mineral Inducers</b>	<b>pDMAEMA</b>	<b>pHPMA</b>
0% HA	PDH0	PHH0
1% HA	PDH1	PHH1
5% HA	PDH5	PHH5
10% HA	PDH10	PHH10
0% LAP	PDL0	PHL0
1% LAP	PDL1	PHL1

5% LAP	PDL5	PHL5
10% LAP	PDL10	PHL10
0 mg/mLALP	PDA0	PHA0
5 mg/mL ALP	PDA5	PHA5
10 mg/mL ALP	PDA10	PHA10

**Table 2-** Nomenclature of all variations of hydrogel.



**Figure S1** – ALP activity kinetics of hydrogels fabricated using ALP enzyme

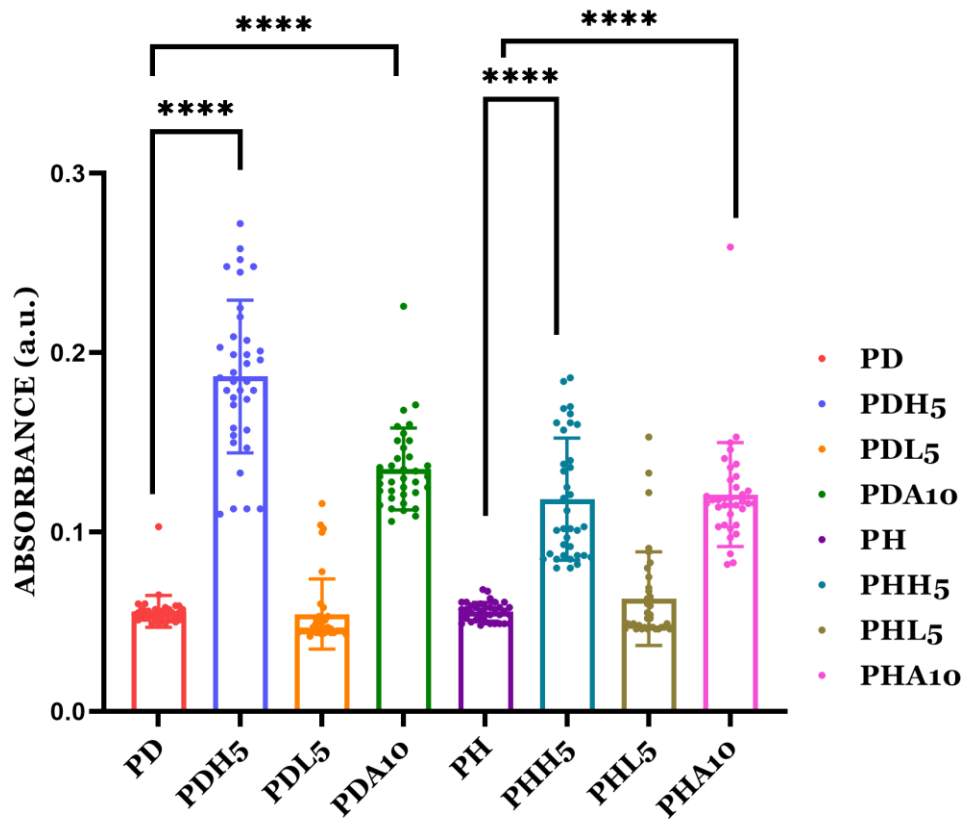


Figure S2 – Alizarin assay of native hydrogels without cells.